The Vitamins

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INTRODUCTION

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The achievements in the science of nutrition which have developed in recent years are among the most significant of all that have been made in modern medical science. The original five letter alphabet for the vitamins has been greatly extended and even the original letters have begun to be subdivided. Vitamin B, instead of being just one or two vitamins, may actually include eight or more different specific principles.

Throughout the world the tendencies of research in vitamins incline largely to efforts which contemplate the isolation of vitamins in pure form and the synthesis of vitamins when possible. At this writing at least nine compounds having the properties of vitamins have been isolated and at least four have been synthesized.

In a previous survey of progress in this field it was found necessary to warn the public and the medical profession against the commercial exploitation of individual vitamin preparations and particularly against the promotion of mixtures of vitamins as panaceas. The Council on Pharmacy and Chemistry is still convinced that for the present there seems to be no more logical basis for including a variety of vitamins in one preparation than there is for combining a number of other well known dietary essentials in a single pharmaceutical product. Since, however, vitamins A and D occur together in nature and particularly since it has been customary now to give them together in the form of cod liver oil or halibut liver oil with added vitamin D, the Council will accept products containing these two vitamins.

The articles which follow represent the views of a considerable number of authorities who have endeavored to summarize the available knowledge concerning certain phases of vitamin study. The average reader will probably be impressed by the fact that studies of the vitamins are concerned not only with the human being but also and particularly with the diets of tional units per gram (or per cubic centimeter), or if it is a natural product which may have been subjected to a process of dehydration.

6. In connection with medicinal foods acceptable for N. N. R., the claim that a food is valuable because of its thiamin content may be made only if it provides in the quantity of food consumed daily at least 200 units of thiamin.

Any food preparation having less than such an amount cannot be regarded as a noteworthy medicinal source of the vitamin. In the light of present knowledge the daily requirement for thiamin appears to be not less than 50 units (International) for the infant and 200 units (International) for the adult.

7. While it has not been established that thiamin deficiency is the sole cause of conditions described as alcoholic neuritis, the neuritis of pregnancy and the neuritis of pellagra, there is some definite evidence of the value of this vitamin in the treatment of these conditions. Vague representations with respect to the value of thiamin in the treatment of other types of neuritis are not permissible.

8. It appears that there is an increased requirement for thiamin when there is greatly augmented metabolism such as occurs in febrile conditions, hyperthyroidism, or vigorous muscular activity.

NICOTINIC ACID AND NICOTINIC ACID AMIDE

Allowable Claims.—1. Nicotinic acid (amide) is recognized as a specific only in the treatment of acute pellagra in relapse. Its administration in appropriate doses leads to the disappearance of all alimentary, dermal, and other lesions characteristic of the disease, to a return to normal of the porphyrin content of the urine, and to a profound improvement in the mental symptoms when the latter are the result of an inadequate intake of nicotinic acid (amide). Nicotinic acid is without influence upon the polyneuritis so frequently observed in pellagrous patients. In such cases it may be necessary to insure the presence in the diet of foods rich in vitamin B_1, or to administer thiamin chloride.

2. Available evidence does not warrant the use of nicotinic acid (amide) for prophylactic purposes, or the suggestion that it be employed as a supplement to the ordinary diet. The protective dose, and the amount which should be present in a well-balanced ration, are unknown.

ASCORBIC ACID (CEVITAMIC ACID)

Allowable Claims.—1. Definite claims for the therapeutic value of ascorbic acid should be permitted only in relation to scurvy until further clinical or experimental evidence has substantiated its usefulness in other states.

2. Ascorbic acid is acceptable for the correction and prevention of scurvy. This effect has been established experimentally and by clinical investigation.

3. It may be permissible under certain conditions to refer to the therapeutic value of ascorbic acid in early and latent scurvy.

Convincing clinical evidence has established that this state does occur. It would be well to emphasize the fact that the diagnosis rests, however, on the basis of roentgenologic evidences in the long bones, and possibly failure to excrete an optimum amount of ascorbic acid in the urine.

4. Dental caries, pyorrhea, certain gum infections, anorexia, anemia, undernutrition and infection alone are not in themselves sufficient indications of ascorbic acid deficiency but according to experimental and clinical investigation they may be concomitant signs of ascorbic acid deficiency. Therefore, it would be permissible to accept the claim for the therapeutic value of ascorbic acid in these symptomatic conditions *only when* it is definitely stated that they are the consequences of a deficiency or suboptimal amount of ascorbic acid or when there is a pathologic interference with assimilation of the amount necessary for the preservation of health.

5. Unless more convincing evidence is present than is now available, no claim referable to the anti-infective effect of ascorbic acid will be recognized. Secondary infections are characteristic of disturbances of nutrition, particularly in all vitamin deficiency diseases. It has not been established that ascorbic acid has a therapeutic effect which directly influences associated secondary infections in scurvy.

6. Because ascorbic acid is a dietary essential, its administration in concentrated form is of value in conditions where difficulty in introducing orally or utilizing ordinary foods in the usual way is encountered. Ascorbic acid is accepted as an essential dietary constituent in infant feeding but it should not be accepted for use in the treatment of diseases except according to the conditions mentioned above. It is generally administered in the form of an ascorbic acid carrying juice. It may be administered parenterally in concentrated form as sodium ascorbate when persistent vomiting, diarrhea, or other conditions prevent the utilization of proper amounts taken orally.

7. Ascorbic acid offered for clinical use must state the potency in terms of the International unit. The International unit for ascorbic acid, which was formerly defined as the vitamin C activity of 0.1 cc. of lemon juice, has now been defined as the ascorbic acid activity of 0.05 mg. of ascorbic acid. This is the quantity of ascorbic acid usually found in 0.1 cc. of lemon juice.

8. In the opinion of the Cooperative Committee on Vitamins, adopted by the Council, the claim that a food is valuable because of its ascorbic acid content should be permitted only if it provides a daily intake of at least 250 units of ascorbic acid.

9. A reasonable general statement regarding allowable claims for ascorbic acid would be as follows:

An optimum amount of ascorbic acid should be supplied at all ages for its therapeutic value in preventing the development of acute or latent scurvy.

Claims for the therapeutic value of ascorbic acid may be accepted when the agent is described as a corrective measure for scurvy due to a demonstrable absence or a suboptimal quantity in the diet, or in cases in which it is definitely known that there is interference with the absorption of an optimal amount.

Advertising of ascorbic acid for such symptoms as failure to gain in weight or stoppage of growth, anorexia, anemia, infections, symptoms referable to the central nervous system or hemorrhagic conditions cannot be accepted unless it is definitely stated that the symptoms are referable to a demonstrable deficiency of ascorbic acid.

The ascorbic acid equivalent or potency in terms of International units should be stated in all dosage claims for ascorbic acid. Ascorbic acid is easily decomposed in the presence of certain other substances; therefore, care should be exercised against administering it (or orange juice) in mixtures, or by such procedure as to render it ineffective.

VITAMIN D

Allowable Claims.—1. Vitamin D is recognized as a specific in the treatment of infantile rickets, spasmophilia (infantile tetany) and osteomalacia, diseases which are manifestations of abnormal calcium and phosphorus metabolism. Vitamin D is valuable in the prevention as well as in the curative treatment of these diseases. Complications such as renal insufficiency or glandular malfunction may preclude normal response to vitamin D therapy. During acute infections, especially of the gastrointestinal tract, vitamin D may prove ineffective because poorly absorbed.

2. Direct exposure of the skin to ultraviolet light from the sun or from artificial sources results in the formation of vitamin D within the organism but the Council cannot recognize statements or implications that vitamin D has all beneficial effects of exposure to sunshine.

3. There is clinical evidence to justify the statement that vitamin D plays an important role in tooth formation and maintenance of normal tooth structure, but there is no warrant for the claim that adequate vitamin D intake will insure normal tooth structure or that adequate vitamin D intake will prevent dental caries.

4. Animal experimentation has shown that correction of an inadequate intake of vitamin D results in the more economical utilization of calcium and phosphorus and also that the undesirable effects of improper ratios of calcium and phosphorus in the diet can largely be overcome by normal intake of vitamin D. The importance of these observations in their application to man is not entirely apparent because of the lack of adequate clinical evidence showing the availability of different forms

of calcium and phosphorus, but it may be stated that vitamin D has a favorable influence on calcium and phosphorus metabolism.

5. The vitamin D requirement appears to be greatest during the period of infancy. Beyond the age of infancy the exact vitamin D requirement of man under any specified conditions is not known but it appears that the requirement during pregnancy and lactation is increased.

6. Clinical evidence does not warrant the claim that massive doses of vitamin D are of benefit in chronic arthritis, in allergic disorders, or in psoriasis.

CHAPTER I

THE CHEMISTRY OF VITAMIN A AND SUBSTANCES HAVING A VITAMIN A EFFECT

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Shortly before the late Dr. Lafayette B. Mendel wrote an article on vitamin A for the previous vitamin symposium in THE JOURNAL, the perplexing question as to why the yellow-red plant pigment carotene exhibits vitamin A activity although the familiar vitamin A of liver oils is essentially a colorless substance had been answered by the discovery [1] that carotene is convertible in the body to vitamin A. In fact the chemical basis for such a relationship had just been established by the brilliant researches of Karrer and his associates, who had determined the chemical constitution both of plant carotene [2] and also of vitamin A [3] from fish liver oil.

The first complete structural formula for carotene (fig. 1) has since turned out to be that of β-carotene, which is by far the most important and widely distributed of the known coloring matters which have vitamin A activity.

β-carotene is a nitrogen-free, aliphatic, methylated polyene having two identical, unsaturated, methylated terminal rings. (The term polyene is given to compounds having a series of conjugated double bonds.) When the aliphatic central chain is examined more closely it may be considered as a condensed chain of four dehydrated isoprene (β-methylbutadiene) residues, shown in the diagram between the broken vertical lines.

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[1] Moore, Thomas: Vitamin A and Carotene: V. The Absence of the Liver Oil Vitamin A from Carotene; VI. The Conversion of Carotene to Vitamin A in Vivo, Biochem. J. **24:** 696 (No. 3) 1930; The Distribution of Vitamin A and Carotene in the Body of the Rat, ibid. **25:** 275 (No. 1) 1931.

[2] Karrer, P.; Helfenstein, A.; Wehrli, H., and Wettstein, A.: Pflanzenfarbstoffe: XXV. Ueber die Konstitution des Lycopens und Carotins, Helv. chim. Acta **13:** 1084, 1930.

[3] Karrer, P.; Morf, R., and Schöpp, K.: Zur Kenntnis des Vitamins-A aus Fischtranen II. Helv. chim. Acta **14:** 1431, 1931.

It will also be noted that these isoprene residues are arranged in pairs, which are united in reverse order at the carbons 15, 15′.

The discovery of this fact by Karrer and his associates is important to an understanding of the relation of carotene to vitamin A because the double bond uniting the two pairs of reversed isoprene residues is evidently capable of hydrolysis giving rise to an alcoholic group in each of the split products. This is the only known basis for the formation of vitamin A from those carotenoids which, in addition to the same polyene chain, also possess, at one or both ends of the structure, the same rings possessed by β-carotene.

In keeping with these facts, Karrer and his associates proposed the structure for vitamin A shown in figure 2, which is now generally accepted.

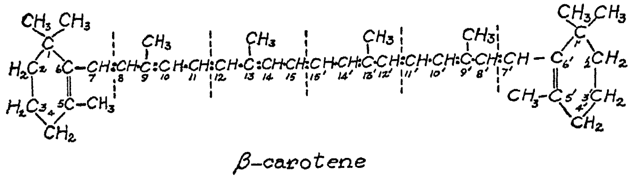

Fig. 1.—Structure of β-carotene.

The ring structure in both β-carotene and vitamin A proved to be of great importance for establishing the structural and the chemical relationships of these substances. The ingenious researches of Karrer were the first to prove that β-carotene contains two β-ionone rings, β-ionone having the structure shown in figure 3.

No chemical designation has yet been adopted for vitamin A analogous to that for other vitamins. The author has suggested elsewhere[3a] that any of the following would be suitable, namely, β-ionone-diisoprenol, semi-β-carotinol, β-ioninol or β-ionin.

When the β-ionone structure was demonstrated in carotene it was relatively easy to demonstrate its occurrence in a highly purified vitamin A preparation from fish liver oil. Soon Heilbron, Morton and Webster[4] were able to substantiate Karrer's formula by forming a naphthalene compound from vitamin A which one could account for only by assuming the correctness of Karrer's formula. Also, Karrer[5] himself completed the proof by an eight step synthesis of crystalline perhydrovitamin A (the completely hydrogenated derivative of the highly unsaturated compound), beginning with *β-ionone, and proving its complete chemical identity* with the similarly crystalline hydrogenated natural vitamin from fish liver oil.

[3a] Outlines of Biochemistry by R. A. Gortner, 2nd Edition, chapter xxxvii, p. 866. John Wiley and Sons, Inc., New York, 1938.

[4] Heilbron, I. M.; Morton, R. A., and Webster, E. T.: The Structure of Vitamin A, Biochem. J. **26**: 1194 (No. 4) 1932.

The final step in this chemical story is the synthesis of the pure vitamin in vitro. This has been accomplished.[6] The isolation of the crystalline natural vitamin has also been reported.[7]

The ring structure in vitamin A is of great biochemical importance. Not only does it play an as yet undetermined role in the biologic activity of the vitamin but it has also been demonstrated that of the thirty or more carotenoids whose chemical structure now seems well established only four may be converted in vivo to vitamin A; likewise, only these possess the optically inactive β-ionone ring. Of these four vitamin A precursors only β-carotene has two such rings; α-carotene has one β-ring and one optically active α-ring; γ-carotene has only one ring, a β-ring, the other potential ring being open, and cryptoxanthin has one β-ring and one β-oxidized ring. γ-carotene, being a monocyclic compound, is related both to carotene and to lycopene, the familiar red tomato pigment, which is a noncyclic hydrocarbon having both potential β-ionone

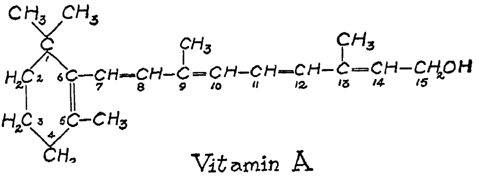

Fig. 2.—Structure of vitamin A.

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[5] Karrer, P.; Morf, R., and Schöpp, K.: Synthese des Perhydrovitamins A, Helv. chim. Acta **16**: 557, 1933.

[6] Fuson, R. C., and Christ, R. E.: The Condensation of β-Cyclocitral with Dimethylacrolein, Science **84**: 294 (Sept. 25) 1936. Kuhn, R., and Morris, C. J. O. R.: Ber. d. Deutsch. chem. Gesellsch. **70B**: 853, 1937. Batty, J. W.; Buraway, A.; Heilbron, I. M.; Jones, W. E., and Lowe, A.: The Synthesis of Vitamin A, J. Chem. Soc. (London) 1937, p. 755.

[7] Holmes, H. N., and Corbet, Ruth E.: A Crystalline Vitamin A Concenctrate, Science **85**: 103 (Jan. 22) 1937. Isolation of Crystalline Vitamin A, J. Am. Chem. Soc. **59**: 2042, 1937.

rings open. As previously stated, β-carotene is much the most important of the four known vitamin A precursors, α-carotene and, especially, γ-carotene and cryptoxanthin having a limited distribution in the natural foodstuffs of man and animals.

In several of the best known and most widely distributed carotenoids, one or both of the terminal rings have one or more alcohol (=CHOH) groups, as already mentioned for cryptoxanthin, or they may have one or more ketone (=CO) groups. Astacin (astacene), the familiar red pigment of lobster and salmon, is a tetraketo-β-carotene. It is a curious fact, however, that the part of a carotenoid molecule possessing the α-ionone ring or its alcohol or ketone derivatives or containing the alcohol or ketone derivatives of the β-ionone ring is not convertible in vivo to vitamin A.

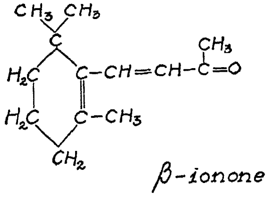

Fig. 3.—Structure of β-ionone.

As carotenoids which have no ring structure, such as lycopene, capsorubin (capsorubene), bixin (bixene) and crocetin (crocetene), have no vitamin A activity, as is the case for one-half the molecule of γ-carotene, previously referred to, it is concluded that the intact β-ionone ring is an essential component of the vitamin A molecular structure. It is further concluded that each molecule of β-carotene is capable of giving rise to two molecules of vitamin A, while α-carotene, γ-carotene and cryptoxanthin may give rise to only one vitamin A molecule. It is further concluded that no other carotenoid[8] whose structure has been determined is a potential vitamin A precursor.

The carotenoids possessing alcohol-containing or ketone-containing ionone rings are much more abundant in nature than are the carotenes. Several of these carotenoids were formerly classed together under the general term xanthophyll because of their characteristic yellow color. Of the many xanthophylls (?) now known, only one is still called xanthophyll, more specifically plant xanthophyll (sometimes called lutein); it is a dioxy-α, β-carotene. Earlier reports of vitamin A activity of crystalline plant xanthophyll were not confirmed and would not have been expected to be confirmed had the chemical relationships been established between the carotenes and vitamin A.

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[8] A possible exception is echinenone, a carotenoid from the sea urchin (Lederer, E., and Moore, T.: Echinenone as a Provitamin A, Nature **137**: 996 [June 13] 1936).

TABLE 1.—*A Partial List of Carotenoids, Their Important Sources and Their Relation to Vitamin A* *

Name	Sources	Molecules of Vitamin A Possible from One Molecule of Pigment
α-carotene	Red palm oil, chestnuts, carrot root, mountain ash berries	1
β-carotene	Green leaves, carrot root, red palm oil, butter	2
γ-carotene	Fruits of Gonocaryum pyriforme (a Dutch East Indies plant), leaves of lily-of-the-valley	1
Cryptoxanthin	Red calix and fruit of the Chinese lantern plant, yellow corn, egg yolk, green grass	1
Xanthophyll (lutein)	Green leaves and grass	0
Zeaxanthin	Yellow corn, green leaves, egg yolk	0
Rhodoxanthin	Seed coat of the yew	0
Astacin	Lobster, salmon, shrimp	0
Lycopene	Red tomato, watermelon	0
Capxanthin	Red peppers, paprika, pimiento	0
Fucoxanthin	Brown algae	..
Taraxanthin	Dandelion, sunflower, cockspur	0
Violaxanthin	Yellow pansy	0
Flavoxanthin	Buttercup	0

* The carotenoids mentioned are not necessarily the exclusive cause of the pigmentation of the products mentioned. In most cases they are not. Of the vitamin A active products, red palm oil and carrots contain chiefly β-carotene and α-carotene and butter chiefly β-carotene, and of the carotenoids in green leaves, β-carotene is found almost exclusively, although xanthophyll and zeaxanthin are the dominant carotenoids.

Most of the pigments formerly classed as xanthophylls now carry a specific name ending in -xanthin, such as zeaxanthin, the dominant pigment of yellow corn, also found in green leaves; capxanthin, the dominant pigment of the red peppers, paprika and pimento; fucoxanthin, the dominant pigment of the so-called brown algae; flavoxanthin, the buttercup pigment; violaxanthin, from the yellow pansy, and taraxanthin, from the dandelion.

Table 1 shows some of the more important carotenoids, their chief occurrence in nature and their relation to vitamin A.

The aliphatic primary alcohol structure of vitamin A is important also in connection with its biochemical properties. It allows for esterification and therefore for compounds of vitamin A with fat acids, proteins and bile acids, which would not be possible for any of the known carotenoid precursors of the vitamin except cryptoxanthin, which, as already stated, has an alcohol group on one of its ionone rings. The alcohol groups on the ionone rings are also capable of ester formation; in fact, the carotenoids which possess such structures apparently exist in nature as esters rather than as free pigment. Various fat acid esters of vitamin A have been described, and there is evidence to indicate that the vitamin is absorbed as bile acid compound,[9] transported in the blood and lymph as fat-acid ester[10] and stored in the liver as a similar compound.[11] This suggests that the vitamin performs its biochemical function in chemical combination with other substances rather than as free substance. Support for this theory is found in the experiments of Wald,[12] which point to a close chemical relationship between the visual purple (rhodopsin) of the retina and a protein-vitamin A compound, which offers a chemical explanation for the well known importance of vitamin A in certain of the visual processes, including its role in the correction of idiopathic hemeralopia.

Vitamin A compounds are decomposed, with the liberation of the vitamin, by such hydrolytic processes as occur in saponification, although the vitamin, being an alcohol, is not itself saponifiable. Most of the carotenoids, as their structure indicates, are also nonsaponifiable. Therefore the vitamin A precursors, together with any vitamin A, appear in the non-saponifiable extracts of fats and oils that contain them. This is the basis of the manufacture of the so-called cod liver oil "concentrates." Because of its alcoholic nature, vitamin A may be separated from its carotenoid precursors by the "phase-separation" test, by which a petroleum ether (low boiling) solution of the mixed products is shaken with 80 to 90 per cent methyl alcohol. The alcohol will extract the vitamin A from the immiscible upper layer of petroleum ether, leaving behind all its carotenoid precursors. Vitamin A esters, however, will, if present in the mixture, remain with the petroleum ether soluble substances.

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[9] According to J. D. Greaves and C. L. A. Schmidt (Relation of Certain Bile Acids to Absorption of β-Carotene in the Rat, Proc. Soc. Exper. Biol. & Med. **36:** 434 [May] 1937), carotene cannot form compounds with bile acids, although the bile is essential for the absorption of the provitamin (The Utilization of Carotene by Jaundiced and Phosphorus-Treated Vitamin A Deficient Rats, Am. J. Physiol. **111:** 502 [April] 1935). See also von Euler, Hans, and Klussmann, Ericka: Zur Biochemie der Carotinoide und des Vitamins C (Ascorbinsäure), Ztschr. f. physiol. Chem. **219:** 215 (Aug.) 1933.

[10] Drummond, J. C.; Bell, Muriel E., and Palmer, Elizabeth T.: Observations on the Absorption of Carotene and Vitamin A, Brit. M. J. **1:** 1208 (June 15) 1935.

[11] Reti, Ladislav: Contribuciones al estudio bioquímico de la vitamina A: I. Sobre el estado de combinación de la vitamina A en las aceites de higado, Rev. Soc. argent. de biol. **11:** 283 (July) 1935; Sur l'état de combinaison de la vitamine A dans les huiles de foie, Compt. rend. Soc. de biol. **120:** 577 (No. 33) 1935. Hickman, K. C. D.: Molecular Distillation. State of the Vitamins in Certain Fish Liver Oils, Ind. Eng. Chem. **29:** 1107, 1937.

[12] Wald, George: Carotenoids and the Visual Cycle, J. Gen. Physiol. : 351 (Nov. 20) 1935.

Consideration must be given the possible importance of the unsaturated bonds in vitamin A. Four of these are present in the aliphatic side chain and one in the ionone ring. Perhydrovitamin A and perhydro-β-carotene, which are completely hydrogenated substances, have no biologic activity, but both α-dihydrocarotene and β-dihydrocarotene are active,[13] as is also di-iodo-carotene. The double bonds eliminated when the last-named by-product is formed are solely in the side chain. However, it is not entirely clear whether the relation of unsaturation to physiologic activity resides solely in the β-ionone ring or whether some unsaturation in the side chain is also important. It is certain of course that the side chain unsaturation explains for the most part the probable function of vitamin A as oxidation reduction catalyst, also possessed by the carotenoids. The unsaturated bonds explain the action of both vitamin A and its precursors as hydrogen acceptors, and experimental evidence has shown that the substances readily absorb oxygen in solution and are markedly pro-oxygenic when undergoing oxidation. Highly oxidized carotenes and vitamin A have no biologic activity, a fact of great importance in the preservation of the vitamin A activity of foods and medicinal products. When the β-carotene molecule is broken at one of the polyene double bonds, using mild oxidation, alcohols are produced which resemble vitamin A structurally although they retain the central structure of the reversed isoprene residues at which hydrolysis occurs to form vitamin A. These so-called apo-carotinols are biologically active and may occur in nature. It is not known whether this biological activity is due to the fact that they are changed to the classical vitamin A in the body or possess physiological activity per se.[13a] Hydroxyphenolic compounds, such as hydroquinone and vitamin E, exert some protective action against the oxidation of carotenes and vitamin A. Thyroxin, diiodotyrosine, tyrosine and adrenalin inhibit the autoxidation of vitamin A.[13] Vitamin A and its precursors are stable to the usual cooking and heat-processing procedures provided they are protected against oxidation.

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[13] von Euler, H.; Karrer, P.; Hellström, H., and Rydbom, M.: Die Zuwachswirkung des isomeren Carotine und ihrer ersten Hydrierungsprodukte, Helv. chim. Acta **14**: 839, 1931.

Vitamin A and its precursors exhibit characteristic differences in their affinity toward various adsorbents, such as calcium carbonate and alumina (aluminum oxide). When a petroleum ether solution of the mixture is filtered through a tightly packed column of finely powdered aluminum oxide, the order of decreasing adsorption affinity will be γ-carotene> cryptoxanthin> β-carotene> α-carotene> vitamin A. This property has been very useful in the isolation and study of the carotenoid precursors of vitamin A.

Each of the vitamin A active carotenoids shows characteristic absorption bands in the visible (blue and violet) region of the spectrum. The exact position of these bands depends on the index of refraction of the solvent employed, and their intensity depends on the molar concentration of carotenoid in the layer through which the light passes. Spectroscopic absorptions of much less intensity occur in the ultraviolet, which may be detected by photographic methods. The wavelengths of maximum absorption of the two most prominent visible bands of the vitamin A active carotenoids in carbon disulfide solution are shown in table 2.

Vitamin A differs from its precursors in showing no absorption bands in the visible region of the spectrum. Instead it shows a rather broad absorption region in the ultraviolet, with a definite maximum at 328 millimicrons in chloroform. These spectroscopic properties form the basis for the spectrophotometric methods for the quantitative estimation of vitamin A and its precursors. The band shown by the blue antimony trichloride compound of vitamin A at 620 millimicrons has also been employed.

[13a] Karrer, P., and Solmssen, U.: β-Carotinal, ein Abbauproduct des β-Carotins, Helv. Chim. Acta **20:** 682, 1937; Karrer, P.; Solmssen, U., and Gugelmann, W.: β-apo-4-carotinal, ein weiteres Abbauproduct des β-Carotins, Helv. Chim. Acta **20:** [illegible], 1937; v. Euler, H.; Karrer, P., and Solmssen, U.: Homologe des Vitamins A (Axerophtols) und ein Abbauproduct des α-Carotins, α-apo-2-carotinal, Helv. Chim. Acta **21:** 211, 1938.

The existence of a second vitamin A, called A_2, has been postulated,[13b] based on the exhibition of a different absorption spectra of the antimony trichloride compound of non-saponifiable extracts which may be obtained from the liver and viscera of various fish other than the cod. The relative distribution of the two forms in various fish has been studied, but the chemical structure of this substance is still unsettled, as is also the biological activity.[13c]

All substances having the polyene structure give color reactions with numerous reagents, most of which are either strong acids or chlorides of polyvalent metals. The intense blue reaction with antimony trichloride given by the carotenes and the blue-violet color given with the same compound by vitamin A have been studied extensively. They serve to reveal the presence of vitamin A and its precursors in animal tissues and products and form the basis of carefully developed quantitative colorimetric methods, especially for preliminary assays of cod liver and other fish liver oils rich in vitamin A, and for exploratory research in the physiology of the vitamin.

TABLE 2.—*Wavelengths of Maximum Absorption of Carotenoids*

Carotenoid	Absorption, Mm.	Maximum, Mm.
γ-carotene	533.3	496.0
β-carotene	521.0	485.0
Cryptoxanthin	518.0	483.0
α-carotene	509.0	477.0

The spectroscopic properties and color reactions mentioned will no doubt be discussed in greater detail in another paper in this series dealing with methods of assay.

13b. Edisbury, J. R.,; Morton, R. A., and Simpkins, G. W.: A Possible Vitamin A_2, Nature (London) **140**: 234, 1937.

13c. (*a*) Edisbury, J. R.; Morton, R. A.; Simpkins, G. W., and Lovern, J. A.: The Distribution of Vitamin A and Factor A_2, Biochem. J. **32**: 118, 1938. (*b*) Lederer, E., and Rathmann, H.: Vitamins A_1 and A_2, Compt. rend. Acad. Sc. **206**: 781, 1938; Gillam, A. E.; Heilbron, I. M.; Jones, W. E., and Lederer, E.: On the Occurrence and Constitution of the 693 *mμ* chromogen (vitamin A_2 [?]) of Fish Liver Oil, Biochem. J. **32**: 405, 1938.

Although the chemical and physiologic relations between vitamin A and its precursor carotenoids have been well established, the conversion of the latter to the former has not yet been accomplished by chemical procedures. All evidence points to the liver as the site of the formation of vitamin A in animals, presumably by means of an enzyme, which has been called carotenase. Earlier reports[14] of demonstration of this action in vitro have been confirmed.[14a] It is clearly unnecessary for man or animals to make this conversion when vitamin A is provided per se in the diet. Indeed, question has been raised as to whether all species[15] are able to form vitamin A from carotene. Marked differences between species no doubt occur with respect to the efficiency of conversion of carotene to vitamin A. It is known that considerable of the ingested carotene occurs in the blood and is deposited in adipose tissue in man, the cow and the horse; in the cow, for which the problem has been studied, a relatively small proportion[16] of ingested carotene is absorbed and converted into vitamin A. Some breeds, such as the Guernsey, convert a smaller proportion of absorbed pigment than others, thus accounting for the larger proportion of carotene than of true vitamin A in their milk fat in contrast to Holstein milk fat, which is lower in carotene but richer in true vitamin A.

Although color is one of the outstanding properties of the vitamin A precursors, in contrast to the essentially colorless vitamin A, some common human foods of animal origin which are not vitamin A precursors are often highly pigmented with carotenoids. For example, the yolk of hen eggs is normally poor in carotene but rich in zeaxanthin and xanthophyll. Normally it contains also vitamin A itself, owing to its formation in the fowl from the vitamin A precursors absorbed from the ration, as well as to the practice of adding fish liver oil to the ration. The carotenoid precursors of the vitamin occurring in egg consist largely of cryptoxanthin,[17] especially when the hen is fed heavily on yellow corn or grass.

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[14] Olcott, H. S., and McCann, D. C.: Carotenase: The Transformation of Carotene to Vitamin A in Vitro, Science **74:** 414 (Oct. 23) 1931; The Transformation of Carotene to Vitamin A in Vitro, J. Biol. Chem. **94:** 185 (Nov.) 1931.

[14a] Wilson, H. E. C.; Ahmad, B., and Mazumdar, B. N.: The Transformation of Carotene into Vitamin A in Liver Antolysates, Indian J. Med. Res. **25:** 85, 1937.

[15] J. L. Rea and J. C. Drummond (Ueber die Bildung von Vitamin A aus Carotin im tierischen Organismus, Ztschr. f. Vitaminforsch. **1:** 177, 1932) obtained evidence that this is not possible in cats.

[16] Bowmann, C. A.; Steenbock, Harry; Beeson, W. M., and Rupel, I. W.: Fat-Soluble Vitamins: XXXIX. The Influence of Breed and Diet of Cows on the Carotene and Vitamin A Content of Butter, J. Biol. Chem. **105:** 167 (April) 1934.

There is much more relation between color and potential vitamin A activity of the common vegetable foods consumed by man than between color and vitamin A activity of animal tissues or products of animal metabolism. Green and yellow (or orange) are the color indicators of potential vitamin A in plants, the former because carotenes always accompany the green chlorophylls. Natural yellow color may be a rough qualitative guide for selection of vitamin A active animal products, because it indicates the consumption of carotenoids and vitamin A precursors by animals even though the carotenoid which colors the product is not a vitamin A precursor. However, all fatty animal products are apt to contain some vitamin A itself if the animal is a transformer of carotene to vitamin A, regardless of the color of the product; some products may be exceptionally rich in true vitamin A and relatively poor in pigment. Certain of the fish liver oils have this characteristic, notably that of the halibut and the burbot.

In connection with any consideration of the relation between color (carotenoid) and vitamin A activity it should be remembered that vitamin A is the only vitamin so far discovered which is a product solely of animal metabolism from precursors which are metabolic products only of plants. This should not be lost sight of in spite of the fact that pure β-carotene is the international vitamin A standard, 1 international unit being the growth-promoting activity in rats of 0.0006 mg. of the standard β-carotene.

For a much more extensive discussion of the chemistry of vitamin A and the substances having the effect of vitamin A than is possible in this article the reader is referred to the extensive paper by Karrer and Wehrli [18] and the monograph of Zechmeister.[19]

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[17] Gillam, A. E., and Heilbron, I. M.: Vitamin A-Active Substances in Egg-Yolk, Biochem. J. **29**: 1064 (No. 5) 1935.

[18] Karrer, P., and Wehrli, H.: 25 Jahre Vitamin-A-Forschung Nova Acta Leopoldina, N. S. **1**: 175, 1933.

[19] Zechmeister, L.: Carotinoide, Berlin, Julius Springer, 1934; Lipochrom und Vitamin A, in Schönfeld, H.: Chemie und Technologie der Fette und Fettprodukte, Vienna, Julius Springer, 1936, vol. 1, p. 147.

CHAPTER II

VITAMIN A

PHYSIOLOGY AND PATHOLOGY

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Vitamin A is an essential part of the biochemical machinery of all vertebrates. It is a derivative of carotenoid pigments from plants. As far as is known, no vertebrate can synthesize carotenoids de novo.[1] Probably all higher animals can convert certain carotenoids to vitamin A. The pathologic consequences of the deficiency are similar in man, monkeys, cattle, swine, dogs, rabbits, guinea pigs, rats, mice and fowls.[2] For these and other reasons, facts about vitamin A learned from animal experiments are without doubt applicable to man.

The required brevity of this article will not permit mention of many papers and excludes critical discussions. The selection and evaluation of the material presented are expressions of our judgment. Readers wishing more references are referred to a monograph by Browning,[3] to a review by Robertson[4] and to the recent volumes of the *Annual Review of Biochemistry.*[5]

PHYSIOLOGY

Vitamin A and Carotenoids.—Fats, carbohydrates and proteins constitute the bulk of the physical basis of living matter and serve as the principal source of energy. Vitamins have to do with those biochemical

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[1] Wald, George: Carotenoids and the Visual Cycle, J. Gen. Physiol. **19:** 351 (Nov.) 1935.

[2] Wolbach, S. B.: The Pathologic Changes Resulting from Vitamin Deficiency, J. A. M. A. **108:** 7 (Jan. 2) 1937.

[3] Browning, E.: The Vitamins, Thomson Research Laboratory, Monograph, London, Baillière, Tindall & Cox, 1931, vol. 1.

[4] Robertson, Elizabeth C.: Recent Work on the Tissue Changes in Vitamin A Deficiency, Am. J. M. Sc. **192:** 409 (Sept.) 1936.

[5] McCollum, E. V.: The Fat Soluble Vitamins, in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press, 1936, vol. 5, p. 379. Sherman, Caroline C., and Sherman, H. C.: Vitamins, ibid., 1937, vol. 6, p. 355. Harris, footnotes 42, 51, 52 and 93.

mechanisms which are concerned with the transformation of food and energy into the many forms of activities essential to normally functioning organisms. Quantitatively the requirement for vitamin A is strikingly small; at most only a few milligrams daily are necessary for the largest mammals.[6]

Carotenoid pigments,[7] yellow and red compounds, are widely distributed in the plant world from bacteria and diatoms to garden fruits and vegetables. Four substances, out of this large group, are known at present from which animals can form vitamin A. These (provitamins) have been named alpha, beta and gamma carotene and cryptoxanthin. (A preparation of a carotenoid, echinenone, from the sex glands of sea urchins, has been found active as a provitamin A.) The chemical nature of these substances has been discussed in a previous paper of this series.[8] The term carotene will be used in this paper to represent the group of provitamins.

Carotene is obviously of great importance in the physiology of plants, though its exact function is unknown.[9] It usually appears concurrently with active growth and function and seems to be closely associated with chlorophyll in assimilative activities. Etiolated shoots contain little carotene while green shoots contain much, a fact that suggests the influence of light in the formation of carotene as well as of chlorophyll. In general, the quantity of chlorophyll is an index of the carotene content of green plants. Carotene does not seem to be consumed by any plant activity carried on in the dark, nor is it lost when the chlorophyll disappears at the time of yellowing of leaves. It is, however, completely destroyed in dry dead leaves. Rapid drying by artificial heat of freshly cut alfalfa destroys enzymes in the plant which in field curing are responsible for extensive inactivation of provitamin A. This fact is important because the carotene of green leaves is brought indirectly into human nutrition through milk and eggs. In human nutrition a considerable part of the vitamin A value, or activity, is represented by carotene in green and yellow vegetables.[10] Eggs, milk and butter are the usual sources of vitamin A but they also furnish some carotene, the proportions depending on the nutritional state of the hen and of the cow.

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[6] Guilbert, H. R., and Hart, G. H.: Minimum Vitamin A Requirements with Particular Reference to Cattle, J. Nutrition **10:** 409 (Oct.) 1935.

[7] Karrer, Paul: Nutritionally Important Natural Pigments, Helvet. chim. acta **19:** E33-E48, 1936.

[8] Palmer, L. S.: The Chemistry of Vitamin A and Substances Having a Vitamin A Effect, J. A. M. A. **110:** 1748 (May 21) 1938.

[9] Sherman, H. C., and Smith, S. L.: The Vitamins, ed. 2, American Chemical Society Monograph, New York, Chemical Catalog Company, Inc., 1931. Zechmeister, L.: Carotinoides in Animal Metabolism, Ergebn. der Physiologie, **29:** 117, 1937.

Except as a precursor of vitamin A, carotene as such is not known to be active in the animal economy. However, the problems of absorption, assimilation and storage of carotene and the relationship of carotene to the secretion of milk and the production of eggs must be considered in any discussion of the physiology of vitamin A.

Storage of Vitamin A in the Body.—Some animals have a remarkable capacity for the storage of vitamin A, illustrated by the fact that a rat may in a few days store enough vitamin A to supply its nutritional needs for several months,[11] while other animals such as the rabbit and guinea pig retain relatively little even when on carotene-rich diets.[11a] Approximately 95 per cent of the total vitamin A in the body of the rat or rabbit is present in the liver; small but detectable amounts appear in the lungs and the kidneys.[12] The distribution of vitamin A depends upon the species. In certain fish the intestines contain larger stores than the liver. In other species the body fat may be an important reservoir.[12a] In times of low intake this store is used to supply physiologic needs. During a prolonged deficiency, the reserve is gradually depleted, normal cell functions are suspended and pathologic change develops. In the rat this storage, physiologically speaking, seems to be of two types. By excess feeding of concentrates, enough vitamin A may be accumulated in the liver theoretically to supply normal requirements for several years. However, this supply decreases at a reasonably rapid rate, beginning immediately after the concentrate is withheld, until it reaches a level at which a more conservative mechanism governs its use. A sufficient supply remains to nourish the animal for some time, even on a deficient diet. In general, the reserves of the liver parallel the dietary intake but may represent only a fraction of the total consumption.[13] In human beings, as in all animals studied, the vitamin A content is much lower in the liver at birth, irrespective of the diet of the mother, than in the liver of the normal adult.[14] Accumulation of vitamin A in the liver tends to increase with age and is dependent on the character of the diet.

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[10] Sherman, H. C.: Chemistry of Food and Nutrition, ed. 5, New York, The Macmillan Company, 1937.

[11] Davies, A. W., and Moore, Thomas: Vitamin A and Carotene: The Influence of the Vitamin A Reserve on the Length of the Depletion Period in the Young Rat, Biochem. J. **31**: 172 (Jan.) 1937.

[11a] Bessey, Otto A.: Unpublished data.

[12] Moore, Thomas: Vitamin A and Carotene: The Distribution of Vitamin A and Carotene in the Body of the Rat, Biochem. J. **25**: 275, 1931.

[12a] Edisbury, J. R.; Morton, R. A.; Simpkins, G. W., and Lovern. J. A.: The Distribution of Vitamin A and Factor A_2, Biochem. J. **32**: 118 (Jan.) 1938. Bessey.[11a]

In the rat, neither general emaciation nor specific starvation due to other vitamin deficiencies has an influence on the hepatic reserves of vitamin A.[15] Chronic diseases and infection seem to lead to slightly lowered vitamin A reserves, probably owing either to reduced intake of vitamin A or to decreased assimilation in the alimentary tract and increased metabolic demands.[16] If one is to judge from autopsy material, nephritis and other diseases of the kidney and bladder definitely lower the vitamin A reserves. As yet the evaluation of data from such material is complicated by the uncertainty of what the normal reserves should be. Even assuming that the reserves are lower than normal, one has the added difficulty of deciding whether the lowering is cause or effect. One might expect greatly decreased reserves in association with severe disease of the liver, but existing data do not allow a conclusion on this point.

The vitamin A concentration of the liver of man,[16] the rat[17] and the cow[6] when the subject is well nourished but not "flooded" with vitamin A is from 200 to 400 international units per gram, or from 10 to 20 mg. per hundred grams. The content of chicken liver seems to be much higher and rabbit and guinea-pig liver lower, under these conditions of stable saturation.[18]

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[13] Davies and Moore.[11] Baumann, Riising and Steenbock.[21]

[14] Ellison, J. B., and Moore, Thomas: Vitamin A and Carotene: The Vitamin A Reserves of Human Infant and Child in Health and Disease, Biochem. J. **31**: 165 (Jan.) 1937. Dann.[40]

[15] Dann, W. J., and Moore, Thomas: The Effect of Vitamin B Deficiency upon the Vitamin A Reserves of the Rat, Biochem. J. **25**: 914, 1931.

[16] Moore, Thomas: Vitamin A and Carotene: The Vitamin A Reserve of the Adult Human Being in Health and Disease, Biochem. J. **31**: 155 (Jan.) 1937.

[17] McCoord, A. B., and Luce-Clausen, Ethel M.: The Storage of Vitamin A in the Liver of the Rat, J. Nutrition **7**: 557 (May) 1934.

[18] Cruickshank, Ethel M., and Moore, Thomas: The Effect of the Administration of Large Amounts of Vitamin A on the Vitamin A Content of the Hen's Egg, Biochem. J. **31**: 179 (Jan.) 1937. Guilbert, H. R., and Hinshaw, W. R.: Vitamin A Storage in the Livers of Turkeys and Chickens, J. Nutrition **8**: 45 (July) 1934.

The problem of the storage of vitamin A in the livers of fish is interesting from both the commercial and the physiologic point of view.[19] Certain species of fish, such as the halibut, yield liver oil of several hundred times the vitamin A potency of the average cod liver oil. As much as 1 per cent of the weight of halibut livers may be vitamin A. It is difficult to understand how this percentage could be stored vitamin derived from food only, especially since the diet of the halibut seems to be quite low in vitamin A. Of course the possibility exists that, although the concentration of the vitamin in the food is low, the large amount eaten, with gradual accumulation and unusual economy, in time leads to large reserves. That the reserves of the Newfoundland cod increase with age is evidence in support of this view. Phytoplankton, small marine algae, serve as the ultimate source of food for most sea animal life and have been found to contain biologically active carotene. The hypothesis is that these small plants are ingested by small crustacea or little fish, which in turn are eaten by larger fish, and so on, until the carotene of microscopic aquatic plant life becomes converted and stored as vitamin A in the livers of the larger fish. One of the points of weakness in this theory is the almost complete freedom from vitamin A or carotene of crustacea and copepods, the main articles of diet of the cod and the halibut. Crustacea and copepods do, however, contain pigments which originated from carotene and which, although not biologically active for a rat, may hypothetically serve as intermediates for synthesis of vitamin A by the fish. Another view could be that fish possess a power not present in mammals of synthesis from simple materials. This is pure speculation and has little experimental evidence to recommend it at present.

Absorption and Utilization.—Absorption and utilization of vitamin A and carotene are affected by the quantity administered and the manner of administration, the influence of other substances, the degree of saturation of the body and the general physiologic conditions of the alimentary tract.[20] The successful use of

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19. Sherman and Smith.[9] Drummond.[34] Harris.[93]

20. Dutcher, R. A.; Harris, P. L.; Hartzler, E. R., and Guerrant, N. B.: Vitamin Studies: The Assimilation of Carotene and Vitamin A in the Presence of Mineral Oils, J. Nutrition 8:269 (Sept.) 1934. McCollum.[5] Baumann, Riising and Steenbock.[21] Clausen.[22] Davies and Moore.[23] Rowntree.[31] Heymann.[32] Harris.[42]

the rat test method for vitamin A activity is ample demonstration that under these conditions amounts near the minimum requirements of either carotene or vitamin A are well utilized in this species. However, as the intake approaches a point which apparently exceeds the ability of the organism to absorb, convert and store, the efficiency decreases. Less than 10 per cent of an intake of vitamin A which is 2,000 times the necessary minimum is stored in the liver of the rat, while amounts which are not excessive but are satisfactory for optimum nutrition lead to 90 per cent storage.[21] Absorption of vitamin A both in the rat and human being reaches a maximum in three to five hours after administration as shown by disappearance from the intestine and rise in the blood level.[22] Under the same conditions carotene reaches a maximum level in the blood in from seven to eight hours. Fecal excretion accounts for only a small portion of the unutilized excess, the rest of which apparently finds other channels of excretion or is destroyed in the intestine or elsewhere in the body.[23] The other chief path of output, the kidney, seems to play no part in disposition of either vitamin A or its precursor unless the organism is literally flooded with vitamin A, in which case the vitamin is absorbed faster than it can be stored or destroyed and some "spilling" probably occurs into all body fluids.[24] This might be cited as evidence for the view that the organism possesses a mechanism for eliminating the unneeded excess by a destructive process, although delayed absorption undoubtedly results in some destruction in the intestine. The presence of bile[25] is not necessary but is helpful for absorption of vitamin A in the rat.

In general the assimilation of carotene is subject to great variations. Small quantities of carotene in oil are utilized under normal conditions to an extent of from 70 to 80 per cent, but it is generally agreed that larger quantities are not as well utilized as vitamin A.[26] This is especially true if the carotene is fed without being dissolved in oil or with low fat diets. Conflicting reports have appeared on the efficiency of utilization of carotene from carrots, spinach, and other food sources, but it is agreed that high fat diets facilitate the absorption of carotene in this form.[26a] A certain amount of normal absorption of fat seems necessary for transportation of carotene across the intestinal wall. On a fat-free diet rats assimilate only a small fraction of the same dose which is completely utilized when 10 per cent fat is included in the diet.[27] The importance of normal fat metabolism for absorption of carotene is further emphasized by the fact that in jaundice or choledochocolonostomized dogs the use of carotene is inhibited.[28] Some carotene is absorbed by rats with internal bile fistulas if bile acids are also fed. This fact suggests that bile acids directly or indirectly play a part in the transportation of carotene across the intestinal epithelium. One should bear in mind the possibility that a deficiency of vitamin A may arise in association with chronic diarrhea, obstruction of the biliary ducts, pancreatic dysfunction, celiac disease and other disorders involving prolonged faulty fat metabolism. In such cases absorption of carotene may be completely inhibited and assimilation of vitamin A greatly reduced.[29]

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[21] Baumann, C. A.; Riising, Blanche M., and Steenbock, Harry: Fat Soluble Vitamins: The Absorption and Storage of Vitamin A in the Rat, J. Biol. Chem. **107:** 705 (Dec.) 1934.

[22] Clausen, S. W.: Limits of the Anti-Infective Value of Provitamin A (Carotene), J. A. M. A. **101:** 1384 (Oct. 28) 1933.

[23] Davies, A. W., and Moore, Thomas: Vitamin A and Carotene: The Distribution of Vitamin A in the Organs of the Normal and Hypervitaminotic Rat, Biochem. J. **28:** 288, 1934. Baumann, Riising and Steenbock.[21] Heymann.[32]

[24] Boller, R., and Brunner, O.: Ueber die Ausscheidung von Vitamin A im Harn, Klin. Wchnschr. **15:** 1106 (Aug. 1) 1936. Davies and Moore.[23] Heymann.[32]

[25] Greaves, J. D., and Schmidt, C. L. A.: On the Absorption and Utilization of Carotene and Vitamin A in Choledochocolonostomized Vitamin A Deficient Rats, Am. J. Physiol. **111:** 492 (April) 1935.

The direct absorption of vitamin A and carotene into the thoracic duct was demonstrated in a case of chylothorax in which it was possible to drain the chyle for study.[30] Reasonably large quantities of vitamin A were returned almost quantitatively in the chyle, while the recovery of equivalent quantities of carotene was far short of quantitative. The carotene seemed to be chemically free, but the vitamin A appeared in a form which indicated that esterification had probably occurred in the intestine, a fact which may be significant in view of its natural high absorbability. Both substances were associated with the nondiffusible fatty phase, as was to be expected.

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[26] Clausen.[22] Heymann.[32]

[26a] Coward, Katherine H.: The Utilization by the Rat of Vitamin A and Carotene Administered in Different Media, Biochem. J. **30:** 1878, 1936. Wilson, H. E. C.; Das Gupta, S. M., and Ahmad, Bashir: Studies on the Absorption of Carotene and Vitamin A in the Human Subject, India J. M. Res. **24:** 807 (Jan.) 1937. Van Eekelen, Marie, and Pannevis, W.: Absorption of Carotenoids from Human Intestine, Nature **141:** 203, 1938.

[27] Ahmad, Bashir: The Fate of Carotene After Absorption in the Animal Organism. Biochem. J. **25:** 1195, 1931.

[28] Greaves and Schmidt, footnotes 25 and 36.

[29] Altschule, M. D.: Vitamin A Deficiency in Spite of Adequate Diet in Congenital Atresia of Bile Ducts and Jaundice, Arch. Path. **20:** 845 (Dec.) 1935.

[30] Drummond, J. C.; Bell, M. E., and Palmer, E. T.: Observations on the Absorption of Carotene and Vitamin A, Brit. M. J. **1:** 1208 (June 15) 1935.

The simultaneous presence of liquid petrolatum shows little effect on the absorption of vitamin A but seriously diminishes the absorption of carotene by competing with the solvent action of natural fats.[31] This emphasizes the desirability of giving liquid petrolatum separately from carotene-containing material.

During and for some time after certain infections, infants show diminished rates of absorption of carotene and vitamin A, which are not attributable to the accompanying fever, because elevated temperatures produced by other means have no such effect.[32] The explanation of this interesting observation is not clear, but the phenomenon seems to be due to decreased intestinal absorption.

Experimental evidence indicates a better absorption of the natural-occurring esters of vitamin A than the free vitamin which results after saponification.[32a]

One may conclude that, under ordinary circumstances of moderate intake, carotene is slightly less efficient than vitamin A but that, when rapid storage of vitamin A is desired or when there is intestinal disturbance, products such as eggs, milk and fish liver oils are preferable to carotene.[22]

Studies of the vitamin A and the carotene content of blood have been difficult because of the lack of satisfactory analytic methods for tissues of such low concentrations.[33] The values for plasma carotenoid seem to be a measure of the difference between the rate of absorption from the intestine and the rate of absorption by the tissues and not an index of nutritional reserves or intake. The vitamin A level in the plasma is subject to great variation even in normal, carefully controlled animals. However, it seems probable that refined methods will show consistent low values for the blood with deficiency, as compared with higher (although variable) values with normal nutrition. The concentration of vitamin A in the blood is independent of the quantity stored in the liver. Hardly enough work has been done on this subject to evaluate the significance of blood determinations.

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[31] Rowntree, J. I.: The Effect of the Use of Mineral Oil upon the Absorption of Vitamin A, J. Nutrition **3:** 345 (Jan.) 1931. Dutcher and others.[20]

[32] Heymann, Walter: Absorption of Carotene, Am. J. Dis. Child. **51:** 273 (Feb.) 1936. Clausen.[22]

[32a] Emmett, A. D., and Bird, O. D.: Comparative Biological Value of Vitamin A as an Alcohol and Ester, J. Biol. Chem. **119:** xxxi (July) 1937. Hickman, K. C. D.: Molecular Distillation. State of the Vitamins in Certain Fish Liver Oils, Indust. Eng. Chem. **29:** 1107, 1937.

[33] Schneider, E., and Widmann, E.: Der Carotin- und Vitamin A-Spiegel im menschlichen Serum, Klin. Wchnschr. **14:** 670 (May 11) 1935. Chesney, Jack, and McCoord, A. B.: Vitamin A of Serum Following Administration of Haliver Oil in Normal Children and in Chronic Steatorrhea, Proc. Soc. Exper. Biol. & Med. **31:** 887 (April) 1934. McCollum.[5] McCoord and Luce-Clausen.[17] Clausen.[22] Ralli and others.[37]

After carotene is absorbed into the thoracic duct it is found in the liver in quantities dependent on the species. When a colloidal aqueous suspension of carotene is injected into the portal or systemic circulation, it is rapidly removed from the blood stream by the reticulo-endothelial system.[34] If sections of the liver are examined immediately after the injection, granules of pigment may be seen in the Kupffer cells. A greater part of the carotene is thus held in the liver, although carotene is held to a lesser extent elsewhere in the animal. The carotene in the Kupffer cells slowly disappears, and the concentration of vitamin A in the liver increases. Possibly the vitamin A of the liver also resides in the Kupffer cells, since livers which have had the parenchymal cells severely damaged by phosphorus poisoning, which supposedly does not harm the Kupffer cells, retain their store of vitamin A, and blocking of the reticulo-endothelial system by injections of certain particles lowers the capacity of the liver for storing vitamin A.[35] That the liver cells are necessary for the conversion of carotene to vitamin A is suggested by the fact that conversion is decreased by phosphorus poisoning[36] and, in certain diseases of cattle which involve the parenchymal cells, carotene remains unconverted in large quantities in the Kupffer cells.

It is important to note that in some cases carotenemia is due to a low ability to convert carotene and not necessarily to a high intake. The rate of transformation of carotene is diminished by diabetes[37] and certain diseases of the liver.

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[34] Drummond, J. C.: The Vitamins, in Harrow, B., and Sherwin, C. P.: Textbook of Biochemistry, Philadelphia, W. B. Saunders Company, 1935.

[35] Lasch, Fritz, and Roller, Dietrich: Ueber den Beeinflussung des Vitamin A- Stoffwechsels der Leber durch Blockade des Reticuloendothels, Klin. Wchnschr. **15:** 1636 (Nov. 7) 1936.

[36] Greaves, J. D., and Schmidt, C. L. A.: The Utilization of Carotene by Jaundiced and Phosphorus Treated Vitamin A Deficient Rats, Am. J. Physiol. **111:** 502 (April) 1935.

The conversion of carotene to vitamin A in vivo is a slow, variable process, demonstrable only after several days, in the rat, rabbit, hen and cow, while there is some question as to whether it occurs at all in the cat, a carnivorous animal.[38] In view of this fact, it is not surprising that attempts to bring about conversion in vitro have been in general unsuccessful.[39] Thus circumstantial evidence suggests the liver as the site of the formation of vitamin A, although no direct proof is available.

Vitamin A and Milk.—The surprisingly low reserves of vitamin A in the newborn[40] make plain the necessity for an adequate intake of vitamin A during infancy. The vitamin A content of milk is therefore important. Since the conversion of carotene to vitamin A in the animal is not always immediate and complete, a certain amount of the vitamin A activity of milk, eggs and other biologic fluids and tissues is due to carotene, the proportion depending on the dietary intake of plant products and the efficiency of the transformation mechanism of the particular animal.[41] It has been shown with cows consuming the same ration that about two thirds of the total activity in Guernsey milk is due to carotene, and this ratio remains rather constant as long as carotene is included in the ration; the carotene content of Ayrshire milk represents only about one third of the total activity, while Jersey milk contains about equal quantities of vitamin A and the provitamin. Thus the proportion of vitamin A activity due to carotene depends on the breed. It is an interesting fact that goat milk contains practically no carotene but that the vitamin A value remains remarkably constant even with considerable variation in rations.

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37. Ralli, Elaine P.; Pariente, A. C.; Brandaleone, Harold, and Davidson, Sidney: Effect of Carotene and Vitamin A on Patients with Diabetes Mellitus: Effect of the Daily Administration of Carotene on the Blood Carotene of Normal and Diabetic Individuals, J. A. M. A. **106:** 1975 (June 6) 1936.

38. Capper, N. S.: Carotene and Vitamin A.: The Transformation of Carotene into Vitamin A as Shown by a Study of the Absorption Spectra of Rat-Liver Oils, Biochem. J. **24:** 980, 1930. Harris.[51] Drummond.[34]

39. Drummond.[34] Harris.[51]

40. Dann, W. J.: Transmission of Vitamin A from Parents to Young in Mammals: Effect of the Liver Reserves of the Mother on the Transmission of Vitamin A to the Foetal and the Suckling Rat, Biochem. J. **28:** 2141, 1934.

41. Baumann, C. A.; Steenbock, Harry; Beeson, W. M., and Rupel, I. W.: Fat-Soluble Vitamins: The Influence of Breed and Diet of Cows on the Carotene and Vitamin A Content of Butter, J. Biol. Chem. **105:** 167 (April) 1934.

One cannot conclude that the more highly pigmented milks necessarily have a higher vitamin A activity, for, although Guernsey milk may contain more carotene than other milks, it also contains less vitamin A, so the differences according to breed in total vitamin A activity seem to be small.

Most animals show a tendency to regulate the vitamin A content of their milk at a level characteristic of the species. In times of excess dietary intake, vitamin A accumulates in the liver and is used to stabilize the vitamin A content of the milk when the intake is less favorable.[10] Although this stabilizing process is not comparable with the efficiency of strict physiologic control, the vitamin A level in milk remains reasonably constant during average dietary variations.[42]

The extent to which the total vitamin A activity of the milk varies with the dietary intake depends on the nutritional state of the animal.[43] When the carotene intake of the cow is increased from a deficient level there is an immediate increase in both carotene and vitamin A in the milk; however, less influence is shown by each increment until a maximum is reached beyond which increased carotene intake does not affect the level of either carotene or vitamin A in the milk.

Cow's colostrum is from ten to one hundred times richer in vitamin A activity than milk, thus providing the calf with a concentrated food during the first few days of life.[44] There is no obvious variation in the vitamin A value of cow's milk with later periods of lactation.[45]

Dann [44] has shown that the combined carotene and vitamin A content of early human milk has from about five to ten times the biologic activity of cow's milk and is not appreciably increased by feeding supplements of cod liver oil to the mother. Other investigators have reported that late human milk contains about the same

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42. Harris, L. J.: Vitamins, in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press, 1933, vol. 2, p. 271. Sherman.[10]

43. Harris.[42] Gillam and others.[45]

44. Dann, W. J.: Transmission of Vitamin A from Parents to Young in Mammals: The Vitamin A and Carotenoid Contents of Human Colostrum and Milk, Biochem. J. **30:** 1644 (Sept.) 1936. Kramer, M. M.; Bain, M. D.; Kunerth, B. L., and Riddell, W. H.: The Vitamin A Value of Colostrum and Milk of Four Cows, Determined by the Single-Feeding Method, J. Agric. Res. **56:** 227, 1938.

45. Gillam, A. E.; Heilbron, I. M.; Ferguson, W. S., and Watson, S. J.: Variations in the Carotene and Vitamin A Values of the Milk Fat (Butter) of Cattle of Typical English Breeds, Biochem. J. **30:** 1728 (Sept.) 1936.

amount of carotene and vitamin A as cow's milk. Human colostrum has from two to three times the biologic activity of early milk.

Vitamin A and Eggs.—Egg and milk formation are analogous biologic functions, and in general the vitamin A value of the egg is subject to the influences previously described for milk.[46] Dark-colored egg yolks are usually richer in vitamin A activity than light-colored yolks; however, pale eggs produced by hens on diets devoid of carotene and xanthophyll but containing cod liver oil are not appreciably lower in vitamin A potency. When the hen is on a deficient diet the vitamin A value of the egg will slowly decrease as the reserves of the liver are depleted, with a concomitant decrease in the production of eggs. Excess feeding of concentrates leads to considerable temporary increase of vitamin A in the egg, but the value soon returns to a more stable level after the removal of the oil, in spite of the still high reserves in the liver.

Vitamin A and Cell Physiology.—One of the earliest functional changes observable in association with vitamin A deficiency is a decreased ability for dark adaptation, nutritional night blindness which responds to vitamin A therapy. The quantity of visual purple present in the retinas which have been dark adapted for varying periods demonstrates that the resynthesis of visual purple in vitamin A deficient animals is abnormally slow. It is possible also to demonstrate experimentally the correlation between the slow rate of regeneration of visual purple and the delayed dark adaptation.[47] Faulty twilight vision in vertebrates occurs with vitamin A deficiency because the mechanism for the formation of visual purple in the rod of the retina is defective.

Wald [48] has recently shown the previously reported high vitamin A value of retinal tissue to be due to vitamin A. He has presented strong evidence that visual purple is a conjugated protein in which vitamin A is a prosthetic group.

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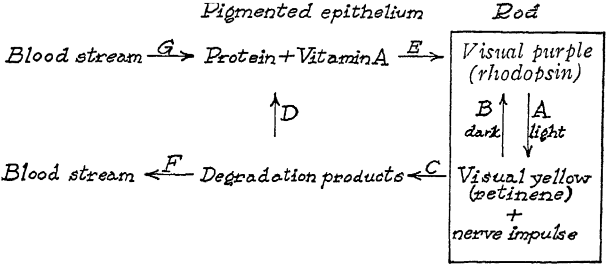
[46] Russell, W. C., and Taylor, M. W.: Vitamin A in Egg and Food, Chem. Abstr. **30**: 529 (Jan.) 1936; Relation Between Vitamin A and D Intake by the Hen and Output in Eggs, J. Nutrition **10**: 613 (Dec.) 1935. Sherman.[10] Cruickshank and Moore.[18]

[47] Fridericia, L. S., and Holm, E.: Experimental Contribution to the Study of the Relation Between Night Blindness and Malnutrition: Influence of Deficiency of Fat-Soluble A Vitamin in the Diet on the Visual Purple in the Eyes of Rats, Am. J. Physiol. **73**: 63 (June) 1925.

[48] Wald, George: Vitamin A in Eye Tissues, J. Gen. Physiol. **18**: 905 (July) 1935. Wald, George, and Clark, Anna-Betty: Sensory Adaptation and Chemistry of the Retinal Rods, Am. J. Physiol. **116**: 157 (June) 1936. Wald.[1]

The cones of the retina are associated with the perceptions of color and other visual functions which depend on light of high intensity, while the rods are sensitive only to light and are especially adapted to function in dim light.[49]

The outside end of the rod which lies adjacent to the pigmented epithelium of the retina contains visual purple (rhodopsin) found only in the rods and serving to transform the energy of dim light into nerve impulses which vary, within limits, with the intensity of the light. Although the complete details of this photochemical process have not been ascertained, it has been suggested and considerable proof obtained that the cycle of events in the retinal rod when a molecule of visual purple receives a quantum of light energy is that shown in the accompanying illustration.[50]



Vitamin A and the perception of light.

The photosensitive pigment (visual purple) absorbs the light and as a consequence is decomposed into products which initiate a nerve impulse (reaction A). Other chemical systems immediately intervene to resynthetize these products to a new molecule of receptor pigment (reaction B or C + D + E) or they are disposed of as waste materials (reaction F) and an entirely new molecule is made from fresh raw material to take their place (reaction G + E). The quantity of visual purple which is present at any instant will depend on the difference between the rate of decomposition (reaction A; intensity of light) and the rate of synthesis (reactions B and E). In bright light the equilibrium of these reactions is displaced so that visual purple is practically absent from the retina, while in the dark the equilibrium is displaced in the opposite direction and visual purple is present in maximum quantity. Normal vision in dim light, whatever may be the details of the reaction, depends on an equilibrium somewhere between these two extremes.

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[49] Tansley, Katharine: Factors Affecting the Development and Regeneration of Visual Purple in the Mammalian Retina, Proc. Roy. Soc., London, s.B **114:** 79 (Nov. 1) 1933. Hecht.[50]

[50] Hecht, Selig: Rods, Cones, and the Chemical Basis of Vision, Physiol. Rev. **17:** 239 (April) 1937. Wald.[1]

Two processes are proposed for the synthesis of visual purple: from visual yellow by simple reversal of photolysis and from the protein plus vitamin A with the aid of the pigmented epithelium. That a certain proportion of visual yellow or retinene is converted to visual purple is observed in the isolated retina which has been bleached and replaced in the dark; the remainder decomposes and is lost or resynthesized by aid of the pigmented epithelium. The latter process takes place in the intact eye but has never been observed in the isolated retina. In vitamin A deficiency, reaction B or E or both are too slow to keep the supply of visual purple adequate.

The fact that the pigmented epithelium is essential to the visual purple process suggests the possibility that it, like other epithelial structures in times of vitamin A scarcity, may not function normally and thus indirectly also contribute toward visual impairment.

The visual purple in fresh water fish differs in spectral characteristics from the rhodopsin found in mammals, birds, amphibia, and marine fish. Wald[50a] has shown this pigment (porphyropsin) to be a component of a retinal cycle which reproduces in behavior and function, but with different components, the features of the rhodopsin system. Both systems appear in the retinas of some fish.

This parallelism has been extended by the observation that vitamin A exists naturally in more than one form.[50b] Vitamin A_2 shows spectral characteristics

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50a. Wald, George: Visual Purple System in Fresh Water Fish, Nature **139:** 1017 (June 12) 1937.

50b. Gillam, A. E.; Heilbron, I. M.; Jones, W. E., and Lederer, E.: On the Occurrence and Constitution of the 693 mμ Chromogen (Vitamin A_2?) of Fish Liver Oils, Biochem. J. **32:** 405 (Feb.) 1938. Lederer, E., and Rathmann, F. H.: Vitamin A_1 and A_2, C. R. Acad. Sc. **206:** 781, 1938.

distinguishable from vitamin A but identical with a carotenoid found as a part of the visual purple systems of fresh water fish. Vitamin A_2 seems to be the principal vitamin A of fresh water fish but also occurs in small quantities in other fish liver oils. It is not known as yet whether this is due to a variability of factors such as food and environment, or whether it represents a fundamental biologic difference in the two classes of fish. Preliminary reports suggest that vitamin A_2 is biologically active for mammals (rat).

The chemistry and the physiology of vitamin A as revealed in its function as a component of visual purple do not necessarily imply the presence of a photosensitive substance in the more generally affected epithelial cells. However, they do suggest that vitamin A may serve as a prosthetic group in other conjugated proteins. The exact nature of these substances and how they function is one of the most interesting problems in cell physiology.

Vitamin A and Other Body Processes.—In association with vitamin A deficiency no significant deviations from the normal have been found for carbohydrate metabolism, blood chemistry, absorption of fat, carbohydrate and proteins, nitrogen metabolism, and calcium and phosphorus metabolism.[51] Fat metabolism does not seem to be obviously altered, as fat is still absorbed, mobilized, desaturated and oxidized during vitamin A deficiency.[52]

Repeated attempts have failed to show any deviations of immunologic mechanisms with vitamin A nutrition which were not due to secondary factors, such as inanition and infection.[53]

PATHOLOGY

The specific pathologic changes of vitamin A deficiency are found in many epithelial structures and may be epitomized as follows: atrophy of the epithelium concerned, reparative proliferation of basal cells, and differentiation of the new product into a stratified keratinizing epithelium. This replacement epithelium, regardless of the previous functions and structure of the region, is identical in all locations and comparable in all its layers with epidermis. Other consequences of vitamin A deficiency are common to other nutritional disturbances, including other vitamin deficiencies.[54]

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[51] Harris, L. J.: Vitamins, in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press, 1932, vol. 1, p. 365.

[52] Harris, L. J.: Vitamins, in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press, 1935, vol. 4, p. 355.

[53] Gellhorn, Ernst, and Dunn, Joan O.: The Influence of Lack of Vitamin A in the Diet on the Phagocytosis Promoting Properties of the Blood Serum, J. Nutrition **13:** 317 (March) 1937. Sherman.[10] Harris, footnotes 42, 51 and 93.

Epithelial Tissues.—Since the pathognomonic pathologic features of vitamin A deficiency are the consequence of epithelial changes, these will be described in some detail. Replacement by keratinizing epithelium is a late effect and follows a nonviable degree of epithelial atrophy. Reparative proliferation on the part of basal cells follows. In epitheliums of the stratified and transitional types, such as those of the cornea, conjunctiva, bladder, ureter and renal pelvis, the basal cells engaged in reparative proliferation may maintain a continuous layer from the beginning, and hence the process appears to be true metaplasia. In other epitheliums, such as those of ducts of glands, the respiratory mucosa and the uterus, where focally distributed basal cells alone have proliferative power, scattered areas of new cells appear. These groups of new (reparative) cells, by their continued growth, undermine, coalesce, and

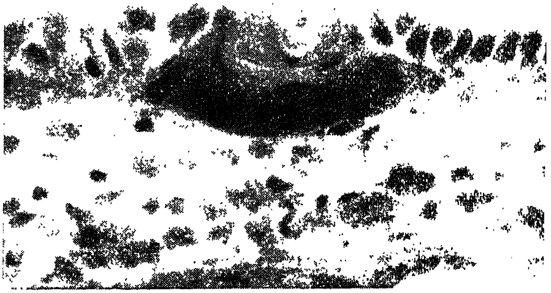

Fig. 1.—This figure shows atrophy of the respiratory epithelium and an early focus of keratinizing metaplasia in vitamin A deficiency, as seen in a section of the mucosa of the turbinate bone of the rat.

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[54] Jackson, C. M.: Recent Work on the Effects of Inanition and Malnutrition on Growth and Structure, Arch. Path. 7: 1042 (June); 8: 81 (July), 273 (Aug.) 1929.

replace the original epithelium by a stratified keratinizing epithelium. Both in experimental animals and in infants, an atrophic epithelium having the histologic appearances of viability may remain for a period above the replacement epithelium. The latter may consist at first of only one or two layers of flat cells, as if the first effort was to cover surface as rapidly as possible. Continued multiplication of cells results in the epidermis-like tissue and separation of the atrophic original epithelium.

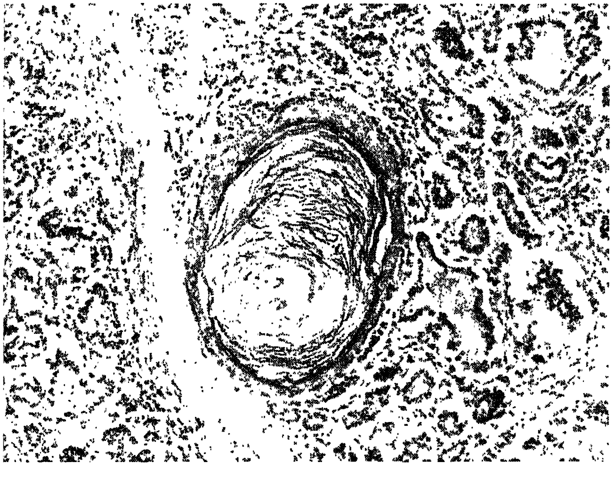

Fig. 2.—Characteristic lesions (keratinizing metaplasia, also accumulation of ductal cells) of vitamin A deficiency as seen in a section of the pancreatic duct in an infant 6 months of age.

Many of the striking gross pathologic features of the deficiency in man and in animals are the outcome of the accumulation of keratinized epithelial cells in glands and their ducts and in other organs. In glands, cysts of considerable size, filled with yellowish cheesy masses of keratinized cells, are formed. In the lungs of human infants as well as of experimental animals this process leads to occlusion of bronchi, formation and filling of bronchiectatic cavities with keratinized cells, and atelectasis. Early workers regarded such cysts as abscesses, and hence vitamin A was believed to protect against infection.

The plugs of desquamated epithelial cells in ducts, bronchi and trachea, which open on regions normally infected with bacteria, act as a culture medium, yet rarely are the surrounding tissues invaded, presumably because of the protection offered by the stratified epithelium.

Distribution of the Keratinizing Metaplasia.[55]—In the rat, the order of response to vitamin A deficiency by keratinizing metaplasia is (1) salivary glands, including the submaxillary, parotid and all accessory glands of the tongue, buccal cavity and pharynx; (2) respiratory tract, including the nares, maxillary sinuses, Jacobson's organ, trachea and bronchi; (3) genito-urinary tract, including the renal pelves, ureters, bladder, epididymis, prostate, seminal vesicles, coagulating glands, uterus, oviducts and accessory sex glands of the vulva, and (4) eyes and parocular glands, including the corneal and palpebral conjunctiva, the harderian, intra-orbital and extra-orbital lacrimal glands and the meibomian glands.

In the guinea pig, lesions of the conjunctiva and parocular glands did not develop in our experiments[56] even though advanced lesions (keratinizing metaplasia and its consequences) were found in all the other locations recorded for the rat. This we now know to have been due to the vitamin A in the orange juice used to prevent vitamin C deficiency. If ascorbic acid is employed for this purpose, the eyes respond late in the course of vitamin A deficiency, though less conspicuously than in rats. Early and extraordinary degrees of metaplasia as compared with this condition in the rat take place in the uterus and bladder of guinea pigs. In both rats and guinea pigs, permanent keratinizing of the vaginal epithelium takes place.

Mason[57] has studied in detail the consequences of vitamin A deficiency on the genital organs of rats.

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[55] Goldblatt, Harry, and Benischek, Maria: Vitamin A Deficiency and Metaplasia, J. Exper. Med. **46**: 699 (Nov.) 1927. Tyson, M. D., and Smith, A. H.: Tissue Changes Associated with Vitamin A Deficiency in the Rat, Am. J. Path. **5**: 57 (Jan.) 1929. Wolbach and Howe, footnotes 56, 58 and 68. Blackfan and Wolbach.[61]

[56] Wolbach, S. B., and Howe, P. R.: Vitamin A Deficiency in the Guinea-Pig, Arch. Path. **5**: 239 (Feb.) 1928.

[57] Mason, K. E., and Wolfe, J. M.: Relation of Castration to Vitamin A Deficiency in the Rat, J. Nutrition **9**: 725 (June) 1935. Mason, K. E., and Ellison, E. T.: The Demonstration of Oestrus in the Vitamin A-Deficient Rat by Supravital Study of the Vaginal Smears, ibid. **10**: 1 (July) 1935; Changes in the Vaginal Epithelium of the Rat After Vitamin A Deficiency, ibid. **9**: 735 (June) 1935. Mason, footnotes 59 and 60.

Atrophy of the testes in the male, noted by Wolbach and Howe,[58] was proved to be constant in animals with vitamin A deficiency even with an adequate supply of vitamin E.[59] In females [60] no injury of the ova, impairment of implantation or disturbances of endocrine function of ovary and anterior hypophysis could be shown. Retardation of growth and death of fetuses due to placental injury were found. Prolonged gestation due to decreased vitality and death of fetuses and difficult parturition with excessive uterine bleeding were other consequences. Resorption of fetal sites occurred with marked deficiencies. The effect on the fetus was shown to be due to placental injury in contrast to vitamin E deficiency when the primary effect is on fetal tissues.

In human infants [61] keratinizing metaplasia has been found in the conjunctiva, cornea, mucosa of the nares, accessory sinuses, including the maxillary antrums, trachea, bronchi, pancreas, renal pelves, ureters, salivary glands, uterus and periurethral glands. The commonest and earliest appearance of the metaplasia is in the trachea and the bronchi. Next in frequency is its appearance in the pelvis of the kidney. The early effect of the deficiency on the respiratory mucosa is a satisfactory explanation of the frequency, severity and persistence of the pneumonia that has been responsible in most instances for the death of infants deficient in vitamin A.[62]

The Eyes.—In the human being, as in experimental animals, involvement of the eye occurs late. The earliest demonstrable change is metaplasia of the epithelium of the conjunctiva and the cornea. Atrophy and metaplasia (of ducts) of the lacrimal glands contribute to the consequences of the accumulation of keratinized cells in the conjunctival sac. The cornea becomes vascularized, edematous and infiltrated with leukocytes. Necrosis of the cornea may occur beneath intact hyperkeratotic epithelium. Infection of the cornea, favored by the accumulation of keratinized cells, may lead to ulceration and hypopyon.

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[58] Wolbach, S. B., and Howe, P. R.: Tissue Changes Following Deprivation of Fat-Soluble A Vitamin, J. Exper. Med. **42**: 753 (Dec.) 1925.

[59] Mason, K. E.: The Specificity of Vitamin E for the Testis: I. Relation Between Vitamins A and E, J. Exper. Zool. **55**: 101 (Jan. 15) 1930.

[60] Mason, K. E.: Foetal Death, Prolonged Gestation, and Difficult Parturition in the Rat as a Result of Vitamin A-Deficiency, Am. J. Anat. **57**: 303 (Sept.) 1935.

[61] Blackfan, K. D., and Wolbach, S. B.: Vitamin A Deficiency in Infants: Clinical and Pathological Study, J. Pediat. **3**: 679 (Nov.) 1933.

[62] Blackfan and Wolbach.[61] Sweet and K'Ang.[73]

The Teeth.—Owing to atrophy and metaplasia of the enamel-forming organ, the continuously growing incisors of rats and guinea pigs are profoundly affected. After atrophy of the enamel-forming organ there are atrophy and cessation or depression of the functioning of odontoblasts. The formation of enamel stops, and striking deformities of the tooth take place because of diminished and defective formation of dentin.[63]

By keeping rats for long periods in a state of incomplete vitamin A deficiency, Orten, Burn and Smith[64] have produced tumor-like formations and tooth duplications at the formative end of the incisors, understandable because of the effect of deficiency on the enamel-forming epithelium. Boyle[65] has described in the tooth germ of an infant with vitamin A deficiency changes in the enamel organ comparable to those occurring in rodent incisors. May Mellanby[66] has summarized her work on teeth in an excellent review and presents evidence that absent or defective enamel and dentin formation are consequences of vitamin A deficiency. Recently May Mellanby and King,[67] presumably on the basis of Edward Mellanby's neurologic observations on vitamin A deficiency, expressed the thought that "loss of neurotrophic control owing to vitamin A deficiency may be partly responsible for pyorrhoea and other diseases of the periodontal tissues and even of dental caries." In all probability, vitamin A deficiency during the formative period of teeth outranks in the human being all other vitamin deficiencies in importance.

The Respiratory Tract.—The consequences of vitamin A deficiency on the respiratory tract can be inferred from the fact that the respiratory mucosa in all locations undergoes keratinizing metaplasia. As atrophy precedes the replacement, clinical effects of the deficiency in all probability occur long before keratinizing epithelium makes its appearance. Death due to pneumonia is common in infants.[62] The pneumonia is usually of the interstitial or peribronchitis type. Regions of atelectasis due to plugging of bronchioles by keratinized cells and bronchiectasis due to cystic dilatation of bronchi from the same cause are gross features of the lungs. The lusterless opaque lining of keratinized cells may be apparent in larger bronchi and tracheas. In the accessory sinuses and nares and probably in the eustachian tubes are other regions where consequences of vitamin A deficiency are obvious.

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[63] Wolbach, S. B., and Howe, P. R.: The Incisor Teeth of Albino Rats and Guinea Pigs in Vitamin Deficiency and Repair, Am. J. Path. **9:** 275 (May) 1933.

[64] Orten, Aline U.; Burn, C. G., and Smith, A. H.: Effects of Prolonged Chronic Vitamin A Deficiency in the Rat, with Special Reference to Odontomas, Proc. Soc. Exper. Biol. & Med. **36:** 82 (Feb.) 1937.

[65] Boyle, P. E.: Manifestations of Vitamin-A Deficiency in a Human Tooth-Germ; Case Report, J. Dent. Research **13:** 39 (Feb.) 1933.

[66] Mellanby, May: The Influence of Diet on the Structure of the Teeth, Physiol. Rev. **8:** 545 (Oct.) 1928.

[67] Mellanby, May, and King, J. D.: Diet and the Nerve Supply to the Dental Tissues, Brit. Dent. J. **56:** 538 (June) 1934.

The Skin.—No noteworthy consequence of vitamin A deficiency in the skin of experimental animals has been described. In Blackfan and Wolbach's cases[68] no cutaneous condition was noted that could be attributed solely to the deficiency. Dryness and scaliness of the skin have been noted, as in malnutrition of diverse causes in infants.

In sexually mature persons, cutaneous lesions associated with vitamin A deficiency and analogous to those occurring in other epithelial structures have been reported by several authors, chiefly from China, but also from South Africa, England and America. The skin becomes dry and rough and then a papular eruption appears. The papules are formed by hyperkeratosis of the hair follicles. Atrophy of sweat glands and keratinizing metaplasia of their ducts are responsible for absence of sweat. Sebaceous glands atrophy and undergo keratinizing metaplasia. The order of appearance and distribution of the papules due to hyperkeratosis of hair follicles is the anterolateral aspect of the thighs and the posterolateral aspect of the upper part of the forearms, extensor surface of the arms and legs, shoulders, abdomen, back, buttocks, the fore part of the neck and the back of the neck. There is a general increase in the pigmentation of the skin and conjunctivae, although in the hyperkeratotic hair follicles and immediately adjacent epidermis the pigment is decreased. Mu, Frazier and Pillat[69] have demonstrated an increase both of melanin-building pigments and of melanin in the darkened skin and conjunctivae.

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[68] Wolbach, S. B., and Howe, P. R.: Epithelial Repair in Recovery from Vitamin A Deficiency: An Experimental Study, J. Exper. Med. **57:** 511 (March) 1933.

[69] Mu, J. W.; Frazier, C. N., and Pillat, A.: Melanin Pigment of the Skin and Conjunctiva in Avitaminosis A in Man, Chinese J. Physiol. **11:** 247 (March 1) 1937.

The best clinical and pathologic descriptions and the complete bibliography are to be found in a recent paper by Frazier and Hu,[70] the first to describe this condition.

The Stomach and Intestine.—Hyperkeratosis with ulceration in the forestomach of rats as a consequence of vitamin A deficiency has frequently been reported. This part of the stomach of the rat is normally lined with stratified squamous epithelium. While hyperkeratosis is of frequent occurrence (we have seen and studied these lesions), it cannot be regarded as specific. 'Pappenheimer and Larimore,[71] who were the first to describe such lesions, excluded vitamin deficiency as a factor. Fuzimaki has reported on unsatisfactory evidence the development of the gastric lesions into carcinoma. That vitamin A deficiency and carcinogenesis are not related has been shown by Sugiura and Benedict.[72]

Lesions of the stomach and intestine in association with vitamin A deficiency are of rare occurrence in man and in experimental animals and, beyond slight degrees of atrophy of the mucosa, are probably not related specifically to the deficiency. The esophagus in man [73] and animals responds by hyperkeratinization. References to gastro-intestinal disturbances in man caused by vitamin A deficiency are given by Robertson.[4]

Genito-Urinary Tract.—In rats complete obstruction of the ureters and renal pelves due to accumulation of keratinized cells may be the cause of death. In human infants [68] less marked degrees of this change are common and may be grossly recognizable post mortem. The formation of calculi in vitamin A deficient rats is probably dependent on an additional factor, as calculi are of extremely rare occurrence in our experience. Keratinization in the urinary bladder occurs in man as well as in laboratory animals. Atrophy of the testes and keratinizing metaplasia of the uterine mucosa may be expected in human adults.

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[70] Frazier, C. N., and Hu, C. K.: Nature and Distribution According to Age of Cutaneous Manifestations of Vitamin A Deficiency: A Study of Two Hundred and Seven Cases, Arch. Dermat. & Syph. **33:** 825 (May) 1936.

[71] Pappenheimer, A. M., and Larimore, L. D.: The Occurrence of Gastric Lesions in Rats: Their Relation to Dietary Deficiency and Hair Ingestion, J. Exper. Med. **40:** 719 (Dec.) 1924.

[72] Sugiura, Kanematsu, and Benedict, S. R.: A Critical Study of Vitamin A and Carcinogenesis, J. Cancer Research **14:** 306 (June) 1930.

[73] Sweet, L. K., and K'Ang, H. J.: Clinical and Anatomic Study of Avitaminosis A Among the Chinese, Am. J. Dis. Child. **50:** 699 (Sept.) 1935.

The Nervous System.—Signs suggestive of nerve lesions in human beings with vitamin A deficiency have not been reported. The experience of investigators has varied in regard to the occurrence of paralysis and other signs of nervous lesions in experimental animals. Hughes, Aubel and Lienhardt[74] in 1928 described extreme incoordination and spasms in swine and found degeneration of myelin sheaths in the spinal cord and in the optic, sciatic and femoral nerves. The same writers[75] also recorded similar signs and lesions in fowls and cattle. In neither report were the affected nerve fibers identified as motor or sensory. Edward Mellanby[76] in 1931 reported incoordination, spasticity and paralysis in dogs nurtured on diets containing much cereal and no vitamin A. Degeneration of myelin sheaths in the spinal cord, particularly in the sensory tracts, was reported.

Seifried[77] in 1932 described ataxia, incoordination and convulsions in fowls. He found degeneration in anterior horn cells of the cord, in ganglion cells of the motor cortex, the nucleus dentatus and nuclei of the medulla, and in the Purkinje cells of the cerebellum. Demyelinization was found in the spinal cord and in the sciatic, brachial and optic nerves. It is to be noted that motor nerve cells were largely involved.

Zimmerman[78] in 1933 described paralysis in rats after very short periods on a vitamin A deficient diet, the majority showing paralysis within forty days. Degeneration of myelin sheaths was found in the brachial plexus and the sciatic nerves but not in the optic nerves. In the spinal cord the lesions were in the sensory tracts and in the crossed and direct pyramidal tracts. Details of the experiment and an account of the signs exhibited by these animals were subsequently reported by Aberle.[79]

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[74] Hughes, J. S.; Aubel, C. E., and Lienhardt, H. F.: The Importance of Vitamin A and Vitamin C in the Ration of Swine, Technical Bulletin 23, Kansas State College of Agriculture, Agricultural Experiment Station, June 1928.

[75] Hughes, J. S.; Lienhardt, H. F., and Aubel, C. E.: Nerve Degeneration Resulting from Avitaminosis A, J. Nutrition **2:** 183 (Nov.) 1929.

[76] Mellanby, Edward: The Experimental Production and Prevention of Degeneration in the Spinal Cord, Brain **54:** 247 (Sept.) 1931.

[77] Seifried, Oskar, cited by Robertson.[4]

[78] Zimmerman, H. M.: Lesions of Nervous System in Vitamin Deficiency: Rats on a Diet Low in Vitamin A, J. Exper. Med. **57:** 215 (Feb.) 1933.

Edward Mellanby[80] in 1934 recorded degeneration of cells of the gasserian ganglion and myelin degeneration of the ophthalmic division of the fifth nerve in rabbits, a dog and rats. He stated, though without presenting his evidence, that degeneration of the afferent nerves is widespread with vitamin A deficiency. The importance of Mellanby's paper lies in his speculation that the epithelial changes of vitamin A deficiency are secondary to loss of neurotrophic control. Similar speculations by May Mellanby[67] in regard to the pathology of teeth have been mentioned. Setterfield and Sutton[81] in 1935 reported degeneration of myelin sheaths in the sciatic and femoral nerves in white rats and claimed superiority for their technic using polarized light on frozen sections of nerves fixed in solution of formaldehyde. Zimmerman and Cowgill[82] confirmed Zimmerman's[78] and Aberle's[79] work and stated that carotene added to the diet prevents the nerve lesions. Carotene, however, did not restore function to the nervous system, even though other evidences of A deficiency were cured. This was attributed to the permanent nature of the nerve lesions.

Failure to observe signs of nerve lesions or to find degeneration of myelin sheaths in vitamin A deficient animals are recorded by an impressive number of workers. Duncan[83] in 1931 reported: "No consistent differences were observed in nerves from animals on diets low in vitamin A or E when compared to nerves from rats of the same age" and "the nerves from healthy well nourished rats exhibited changes equal to those in rats extremely emaciated and paralyzed from the effects of feeding on a diet low in vitamin B."

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[79] Aberle, S. B. D.: Neurological Disturbances in Rats Reared on Diets Deficient in Vitamin A, J. Nutrition **7:** 445 (April) 1934.

[80] Mellanby, Edward: Xerophthalmia, Trigeminal Degeneration and Vitamin A Deficiency, J. Path. & Bact. **38:** 391 (May) 1934.

[81] Setterfield, H. E., and Sutton, T. S.: The Use of Polarized Light in the Study of Myelin Degeneration: The Degeneration of Myelinated Nerves in Avitaminosis A in the White Rat, J. Nutrition **9:** 645 (June) 1935.

[82] Zimmerman, H. M., and Cowgill, G. R.: Lesions of the Nervous System in Vitamin Deficiency: The Effect of Carotene in the Treatment of the Nervous Disorder in Rats Fed a Diet Low in Vitamin A, J. Nutrition **11:** 411 (May) 1936.

[83] Duncan, Donald: The Marchi Method: A Discussion of Some Sources of Error and the Value of This Method for Studying Primary Changes in the Myelin Sheath, Arch. Neurol. & Psychiat. **25:** 327 (Feb.) 1931.

Suzman, Muller and Ungley[84] found no nervous lesions in adult dogs on a diet deficient in vitamin A. Grinker and Kandel[85] failed to observe nervous symptoms in monkeys fed on a vitamin A deficient diet and found no degenerative lesions of myelin sheaths in the central nervous system. Arthur Weil of Chicago and Charles Davison of New York, in the discussion of Grinker and Kandel's paper, reported failures to find nerve lesions in rats in experiments with vitamin A deficiency.

Radhakrishna Rao,[86] in studies with rabbits, rats and fowls, although often finding myelin degeneration in the afferent nerves of the eye, failed to correlate the degeneration with the onset and course of the ocular lesions. Repair of the corneal lesions following administration of vitamin A took place without recovery of the nerve lesions. He concluded that "there is no evidence to suggest that xerophthalmia is secondary to a loss of the neurotrophic control of the ophthalmic division of the trigeminal nerve."

Eveleth and Biester[87] maintained that "neither vitamins A nor B complex are responsible for the myelin degeneration of the spinal cords and peripheral nerves in swine."

Wolbach and Howe[58] in 1925 stated: "As the rats exhibited no symptoms pointing to lesions of the nervous system, the peripheral nerves were not studied. No lesions were found in brain, cerebellum and sympathetic ganglia." Marchi preparations were not made. A review of their records show that a few rats, shortly before death, showed extreme muscular weakness and did drag their hind legs. This was attributed to extreme degeneration of the muscles. In work now in progress, under a grant from the Milton Fund to the Harvard Dental School, in which several types of diets and different stocks of rats are being used, it has become evident that the appearance of nerve lesions is dependent upon more than one factor. Studies of the nervous system made by R. L. Swank [88] with his very reliable modification of the Marchi technic failed to show degeneration of myelin in the spinal cord or peripheral nerves in rats and guinea pigs which had exhibited extreme keratinizing metaplasia of epitheliums.

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[84] Suzman, M. M.; Muller, G. L., and Ungley, C. C.: An Attempt to Produce Spinal Cord Degeneration in Dogs Fed a High Cereal Diet Deficient in Vitamin A: The Incidental Development of a Syndrome of Anemia, Skin Lesions, Anorexia and Changes in the Concentration of Blood Lipoids, Am. J. Physiol. **101**: 529 (Aug.) 1932.

[85] Grinker, R. R., and Kandel, Ernestine: Experimental Vitamin (A, B_1, B_2 and B Complex) Deficiency: Histologic Changes in the Central Nervous System, Arch. Neurol. & Psychiat. **30**: 1287 (Dec.) 1933.

[86] Radhakrishna Rao, M. V.: Studies on Vitamin A Deficiency: I. Xerophthalmia and Trigeminal Nerve Degeneration, Indian J. M. Research **24**: 439 (Oct.) 1936.

[87] Eveleth, D. F., and Biester, H. E.: The Significance of Myelin Sheath Degeneration and Its Relation to Incoordination, Am. J. Path. **13**: 257 (March) 1937.

There is no substantial evidence that degeneration of myelin sheaths is a specific consequence of vitamin A deficiency. That it does occur in some strains of laboratory animals cannot be denied. The relation of degeneration of skeletal muscle to the signs regarded as of nervous origin is a problem having a bearing on the subject. Severe degeneration of muscle is common in association with vitamin A deficiency in man, guinea pigs and rats.[89]

We have observed degeneration of skeletal muscles in guinea pigs deficient in vitamin C and in rats deficient in vitamin B_2 complex. Goettsch and Pappenheimer [90] have described a "progressive highly selective and ultimately fatal dystrophy of the voluntary muscles" in guinea pigs and rabbits on diets with adequate vitamin content. No significant lesions were found in the spinal cord and peripheral nerves. The same authors, with Rogers,[91] could find no alterations of the motor nerve terminals in muscle. It is not clear whether Marchi preparations were employed, as emphasis was placed on silver impregnation methods. These papers by Pappenheimer and his associates are pertinent to the present discussion because they indicate the possibility of ascribing to nerve lesions behavior of laboratory animals caused by muscle degeneration.

Since degeneration of myelin sheaths is not a constant accompaniment of vitamin A deficiency, as is epithelial metaplasia, it is not necessary to present the many objections to Mellanby's thesis that loss of nerve function may be the cause of the epithelial changes which are invariably the consequence of vitamin A deficiency regardless of species.

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[88] Swank, R. L., and Davenport, H. A.: Marchi's Staining Method: Studies of Some of the Underlying Mechanisms Involved, Stain Technol. **9:** 11 (Feb.) 1934.

[89] Wolbach and Howe, footnotes 56 and 58. Blackfan and Wolbach.[61]

[90] Goettsch, Marianne, and Pappenheimer, A. M.: Nutritional Muscular Dystrophy in the Guinea Pig and Rabbit, J. Exper. Med. **54:** 145 (Aug.) 1931.

[91] Rogers, W. M.; Pappenheimer, A. M., and Goettsch, Marianne: Nerve Endings in Nutritional Muscular Dystrophy in Guinea Pigs, J. Exper. Med. **54:** 167 (Aug.) 1931.

Other consequences of vitamin A deficiency, which are not specific, are (1) inability to store fat, (2) hemosiderosis apparently due to anemia, (3) cessation of growth of bones, (4) the degenerative lesions of skeletal muscles already mentioned and (5) lymphoid hypoplasia of the spleen.

Loss of fat occurs in spite of an abundant supply, but this takes place with other vitamin deficiencies, notably deficiency of the B_2 complex. The anemia in infants and experimental animals is accompanied by hemosiderosis in the spleen and liver and, finally, by atrophy of the spleen and bone marrow. In animals, restoration of vitamin A to the diet causes regeneration of bone marrow, disappearance of hemosiderin from the spleen and liver and, in the rat and the guinea pig, active hyperplasia of the spleen and an outburst of erythroblastic activity. The anemia and hemosiderosis also occur with B_2 complex deficiency and with prolonged partial vitamin C deficiency.

The effect of vitamin A deficiency on growth of bone is complete cessation of endochondral bone formation, indistinguishable from that occurring in any long-continued state of malnutrition.

While Mason[60] has studied some of the consequences of vitamin A deficiency in the rat on gestation and parturition, the importance of further research is indicated by the brief publication of Hale[92] recording in the offspring of vitamin A deficient swine various congenital defects, including defective eyes, absence of eyes, misplaced kidneys, harelip, cleft palate and extra earlike growths.

Excessive administration of vitamin A concentrates to experimental animals has been reported to result in damage to bone, kidneys, skin and other soft tissue. However, conclusive evidence awaits a preparation of pure material. No injury is likely by use of the usual liver oils even in high dosage. Large doses of carotene given orally or by injection are innocuous.[93]

Repair in Recovery from Vitamin A Deficiency.—Repair after restoration of vitamin A to the diet is rapid. In rats there is a lag of a few days, but within six to eight days gain in weight, resumption of growth in bones and teeth, and regeneration of bone marrow and spleen are apparent. Reparative changes in the metaplastic epithelium begin as early as the fifth day.[68] The initial changes are separation of superficial keratinized cells and vacuolization of cells of intermediate layers. The epithelium becomes divided into two zones in consequence of progress of vacuolar degeneration and leukocytic infiltration. All those cells, apparently irreversibly differentiated toward keratinization, undergo degeneration; the deep zone, corresponding to the stratum germinativum of epidermis, survives, and the cells proceed to differentiate into the type of epithelium originally present. On the whole the change is an abrupt one and affords further evidence that the epithelial changes of vitamin A deficiency are primary and not of nervous origin, particularly as Zimmerman and Cowgill[82] failed to restore function to the nervous system by administering carotene, while all other manifestations of the consequences of their vitamin A deficient diet were cured.

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[92] Hale, Fred: The Relation of Vitamin A to Anophthalmos in Pigs, Am. J. Ophth. **18**: 1087 (Dec.) 1935.

[93] Harris, L. J.: Vitamins, in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press, 1934, vol. 3, p. 275.

The changes in the repair of epitheliums after vitamin A deficiency have their normal counterpart in changes in the vagina of rodents during the part of the estrous cycle in which the cornified vaginal epithelium returns to the mucous type.

CHAPTER III

THE PHARMACOLOGY AND THERAPEUTICS OF VITAMIN A

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Treatment with vitamin A is indicated in disorders clearly due to deficiency of vitamin A in the diet. These disorders include nutritional night blindness, xerophthalmia, keratomalacia and a definite kind of follicular keratosis of the skin. The use of vitamin A in the prophylaxis of these disorders is also well established. It is appropriate to discuss these well defined uses of vitamin A before undertaking an analysis of the other uses proposed for vitamin A. Tests for loss of adaptation to dim light have been introduced recently as a measure of deficiency of vitamin A. These tests are considered in the section of this review dealing with night blindness.

Treatment with vitamin A has been suggested in conditions obviously not due to deficiency, for example, in hyperthyroidism, because experiments with animals demonstrate an antagonism between vitamin A and thyroxine. Vitamin A has also been used for the local treatment of injuries of the skin because of a supposed stimulating effect on epithelial growth. Vitamin A has been used in conditions which might be associated with deficiency of a moderate degree, but almost without exception the observers provided no evidence of the existence of the moderate deficiency. These conditions include urinary lithiasis and degenerative changes in the nervous system. As a rule, the evidence of benefit from the use of vitamin A in these conditions has been conflicting. The experiments with animals have not given uniformly positive results because the conditions of the experiments have been too varied. Although it cannot be concluded that deficiency of vitamin A is the sole cause of nervous degeneration or of lithiasis, it may be

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admitted that these abnormal conditions may be due to faulty diet, and there is hope that the exact dietary causes will be disclosed eventually.

Most observers find that severe deficiency of vitamin A is accompanied by decrease of resistance to infection. Vitamin A has been rather widely advocated to increase resistance to infection. The clinical evidence often fails to meet the requirements of statistics. Few of the observers have attempted to demonstrate the presence of vitamin A deficiency in their subjects.

Injury to animals results from large doses of vitamin A. These doses are far larger than any proposed for human beings. Ordinary doses of cod liver oil may be harmful to animals consuming unusual diets, especially to herbivora. The injurious effects, at least in herbivora, are not due to the presence of vitamin A in the oil. Clinical experience would indicate that ordinary doses of fish liver oils are not injurious to human beings.

VITAMIN A AND THE EYE

Night Blindness.—Loss of visual acuity in dim light is known as night blindness. It occurs in various diseases of the eye such as toxic amblyopia, retinitis pigmentosa and detachment of the retina, but a form was known in the time of ancient Egypt which could be cured by a diet of liver. In more recent times, night blindness has been reported in prisoners, in Negro slaves in Brazil, in sailors on long voyages, in soldiers during the World War, and in Orthodox Russians during the Lenten fasts. Blegvad [1] found it in thirty-seven Danish cases of xerophthalmia. Ishihara [2] cured it with cod liver oil. Bloch [3] suggested that it is due to deficiency of vitamin A, which, in 1921, on purely clinical grounds, he distinguished from the antirachitic factor. Parinaud [4] suggested that visual adaptation to dim light is a function of visual purple in the retina. Because of these observations, Fridericia and Holm [5] and

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1. Blegvad, O.: Xerophthalmia, Keratomalacia and Xerosis Conjunctivae, Am. J. Ophth. **7**: 89 (Feb.) 1924.
2. Ishihara, S.: Zur Aetiologie der idiopathischen Hemeralopie bzw. Xerosis conjunctivae, Klin. Monatsbl. f. Augenh. **51**: 596 (Jan.-June) 1913.
3. Bloch, C. E.: Clinical Investigation of Xerophthalmia and Dystrophy in Infants and Young Children (Xerophthalmia et Dystrophia alipogenetica), J. Hyg. **19**: 283 (Jan.) 1921.
4. Parinaud, M.: L'héméralopie et les fonctions du pourpre visuel, Compt. rend. Acad. d. sc. **93**: 286 (July) 1881.
5. Fridericia, L. S., and Holm, E.: Experimental Contribution to the Study of the Relation Between Night Blindness and Malnutrition: Influence of Deficiency of Fat Soluble A-Vitamin in the Diet on the Visual Purple in the Eyes of Rats, Am. J. Physiol. **73**: 63 (June) 1925.

Holm[6] studied the formation of visual purple in the retinas of rats which received diets deficient in vitamin A. They found that the rate of regeneration of visual purple after its bleaching by a bright light in the living animal was less in the rats receiving the deficient diet than in the controls. The rats with deficient diet were also night blind. Tansley[7] confirmed these observations. Yudkin, Kriss and Smith[8] showed that the fat extracted from the retina is one of the richest sources of vitamin A and suggested that visual purple may bear a direct structural relationship to vitamin A. Wald[9] has shown that, in mammals, birds, amphibians and certain marine fish, vitamin A unites in the retina with a protein to form visual purple. This process takes place continuously and depends on a sufficient supply of vitamin A. Under the influence of light, visual purple is changed to visual yellow; a portion of the visual yellow reverts, in the dark, to visual purple; another portion undergoes decomposition into vitamin A and a protein. It is apparent that vitamin A takes part in a cycle of changes in the retina and that the rate of formation of visual purple after exposure of the retina to bright light depends on the available supply of vitamin A. The chemical processes involved in vision are the subjects of active study at the present time (Mirsky,[10] Hecht,[11] Dartnall and Goodeve,[12] the von Jancsós[13]).

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6. Holm, E.: Demonstration of Hemeralopia in Rats Nourished on Food Devoid of Fat-Soluble A-Vitamin, Am. J. Physiol. **73**: 79 (June) 1925.

7. Tansley, Katharine: The Regeneration of Visual Purple: Its Relation to Dark Adaptation and Night Blindness, J. Physiol. **71**: 442 (April) 1931.

8. Yudkin, A. M.; Kriss, Max, and Smith, A. H.: Vitamin A Potency of Retinal Tissue, Am. J. Physiol. **97**: 611 (July) 1931.

9. Wald, George: Vitamin A in Eye Tissues, J. Gen. Physiol. **18**: 905 (July) 1935; Carotenoids and the Visual Cycle, ibid. **19**: 351 (Nov.) 1935; The Chemistry of the Visual Purple System, Cold Spring Harbor, Biol. Lab. Symposia on Quant. Biol. **3**: 251, 1935; The Visual Purple System in Marine Fishes, Nature **136**: 913 (Dec. 7) 1935; Pigments of the Retina: I. The Bull Frog, J. Gen. Physiol. **19**: 781 (May 20) 1936; Pigments of the Retina: II. Sea Robin, Sea Bass, and Scup, ibid. **20**: 45 (Sept.) 1936; Bleaching of Visual Purple in Solution, Nature **139**: 587 (April 3) 1937; Visual Purple System in Fresh Water Fishes, ibid. **139**: 1017 (June 12) 1937.

10. Mirsky, A. E.: The Visual Cycle and Protein Denaturation, Proc. Nat. Acad. Sc. **22**: 147 (Feb.) 1936.

11. Hecht, Selig: Rods, Cones and the Chemical Basis of Vision, Physiol. Rev. **17**: 239 (April) 1937. Hecht, Selig; Haig, C., and Chase, A. M.: Influence of Light Adaptation on Subsequent Dark Adaptation of Eye, J. Gen. Physiol. **20**: 831 (July) 1937.

12. Dartnall, H. J. A., and Goodeve, C. F.: Scotopic Luminosity Curve and the Absorption Spectrum of Visual Purple, Nature **139**: 409 (March 6) 1937.

13. von Jancsó, N., and von Jancsó, H.: Fluoreszenzmikroskopische Beobachtung der reversiblen Vitamin-A-Bildung in der Netzhaut während des Sehaktes, Biochem. Ztschr. **287**: 289 (Oct.) 1936.

The facts just presented, together with the observation that night blindness is an early symptom of deficiency of vitamin A, form the basis of recent tests for visual adaptation in dim light as methods for the diagnosis of vitamin A deficiency. Hart, Mead and Guilbert[14] observed spontaneous night blindness in cattle during a severe drought and proved that the visual defect was due to the very low carotene content of the food. By means of accurate clinical observations of the symptom night blindness and by analyses of the organs and of the rations for vitamin A and for carotene, their subsequent studies[15] have shown that night blindness occurs only when the stores of vitamin A in the liver are practically exhausted; the daily requirement of carotene for cattle, sheep and swine depends only on the weight of the animals and does not depend on the rate of growth, the dose being from 25 to 39 micrograms per kilogram of body weight. This amount agrees closely with that found for the rat (25 micrograms per kilogram daily) by Baumann, Riising and Steenbock.[16] When vitamin A is given as cod liver oil, the requirements are the same for the different species of animal and depend only on the weight. The authors give provisional figures of from 5.6 to 8.4 micrograms per kilogram of body weight for the daily requirement of vitamin A.

Studies of dark adaptation in children have been carried out by Jeans and his colleagues,[17] who used at first the Birch-Hirschfeld photometer and later an improved photometer. In their method the subject is placed in the dark for ten minutes and then exposed to bright light for three minutes. During the following

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14. Hart, G. H.; Mead, S. W., and Guilbert, H. R.: Vitamin A Deficiency in Cattle Under Natural Conditions, Proc. Soc. Exper. Biol. & Med. **30:** 1230 (June) 1933.

15. Guilbert, H. R., and Hart, G. H.: Minimum Vitamin A Requirements with Particular Reference to Cattle, J. Nutrition **10:** 409 (Oct.) 1935. Guilbert, H. R.; Miller, R. F., and Hughes, E. H.: The Minimum Vitamin A and Carotene Requirement of Cattle, Sheep and Swine, ibid. **13:** 543 (May) 1937.

16. Baumann, C. A.; Riising, B. M., and Steenbock, Harry: Fat Soluble Vitamins: XL. The Growth Method of Determining Vitamin A, Ztschr. f. Vitaminforsch. **3:** 81 (April) 1934.

17. Jeans, P. C.: Vitamin Deficiency in Childhood, Minnesota Med. **16:** 688 (Nov.) 1933. Jeans, P. C., and Zentmire, Zelma: Prevalence of Vitamin A Deficiency Among Children in Iowa, Am. J. Dis. Child. (Society Transactions) **48:** 928 (Oct.) 1934; A Clinical Method for Determining Moderate Degrees of Vitamin A Deficiency, J. A. M. A. **102:** 892 (March 24) 1934; The Prevalence of Vitamin A Deficiency Among Iowa Children, ibid. **106:** 996 (March 21) 1936. Jeans, P. C.; Blanchard, Evelyn, and Zentmire, Zelma: Dark Adaptation and Vitamin A, ibid. **108:** 451 (Feb. 6) 1937.

ten minute period of recovery, tests for visual acuity are made. The subject must be able to cooperate by stating accurately what he sees. Normal standards were obtained by testing persons known to have been eating diets rich in vitamin A. Jeans reported in 1933 that 25 per cent of rural children and 53 per cent of city children showed poor visual adaptation; later, in a smaller series, about the same incidence was found. The administration of cod liver oil or of carotene equivalent to from 5,000 to 6,000 international units of vitamin A daily brought about recovery in from four to six weeks. The minimum daily requirements for vitamin A in two boys 11 years of age were found to be about 3,000 units. Snelling at first [18] agreed with the results of Jeans but later [19] encountered technical difficulties in using the Birch-Hirschfeld apparatus and stated his belief that experience with the test by the subject might account for some of the "improvement" in readings following the use of cod liver oil. Palmer and Blumberg [20] have presented convincing evidence that improvement in visual acuity with repetition of the test may be due to practice and that variations among normal children are so great that the results of single tests are unreliable. Park [21] has used the method with adults but gives impressions rather than statistics. Jeghers [22a] calls attention to the prevalence and the importance of night blindness in drivers of automobiles. He has also reviewed [22b] the literature on night blindness as a criterion of vitamin A deficiency and has added [22c] observations on adults, using the method of Jeans. Isaacs, Jung and Ivy [23] have failed to find any correlation between the intake of vitamin A and the readings made with the instrument used by Jeans. They subjected their data to rigorous statistical analysis.

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[18] Snelling, C. E.: Vitamin A Deficiency in Children, Am. J. Dis. Child. (Society Transactions) **51:** 484 (Feb.) 1936.

[19] Snelling, C. E.: A Study of the Birch-Hirschfeld Photometric Test for Vitamin A Deficiency, J. Pediat. **9:** 655 (Nov.) 1936.

[20] Palmer, C. E., and Blumberg, H.: The Use of a Dark Adaptation Technic (Biophotometer) in the Measurement of Vitamin A Deficiency in Children, Pub. Health Rep. **52:** 1403 (Oct. 8) 1937.

[21] Park, I. O.: Preliminary Observations on Vitamin A Deficiency as Shown by Studies with the Visual Photometer, J. Oklahoma M. A. **28:** 357 (Oct.) 1935; Further Observations on Vitamin A Deficiency as Shown by Studies with the Visual Photometer and Clinically, ibid. **29:** 129 (April) 1936; Significance of Vitamin A Deficiency, editorial, Am. J. Digest. Dis. & Nutrition **3:** 193 (May) 1936.

[22] (*a*) Jeghers, Harold: Night Blindness Due to Vitamin A Deficiency: Consideration of Its Importance in Traffic Problems, New England J. Med. **216:** 51 (Jan. 14) 1937; (*b*) Night Blindness as a Criterion of Vitamin A Deficiency: Review of the Literature with Preliminary Observations of the Degree and Prevalence of Vitamin A Deficiency Among Adults in Both Health and Disease, Ann. Int. Med. **10:** 1304 (March) 1937; (*c*) The Degree and Prevalence of Vitamin A Deficiency in Adults, with a Note on Its Experimental Production in Human Beings, J. A. M. A. **109:** 756 (Sept. 4) 1937.

Edmund and Clemmesen [24a] have used as a criterion for loss of visual acuity in dim light the power of distinguishing simple geometric forms. Friderichsen and Edmund [24b] use for their criterion the faintest illumination to which a child reacts when his eye is fully adapted to darkness. The investigator observes reflex movements—squeezing together of the lids, oculomotor movements, turning the head toward the light. The authors make the surprising statements that improvement in visual acuity may occur within half an hour after the administration of halibut liver oil and that in some cases in which improvement has not occurred after administration of cod liver oil or halibut liver oil prompt improvement occurs after the administration of a preparation of spinach. Their method does not determine the rate at which visual purple is regenerated in light or in darkness as does that of Jeans, but it has the advantage that it can be applied to infants.

It is evident that the use of the methods described requires a certain amount of practice. Since they apparently reveal an incidence of night blindness far greater than any one had supposed might exist, confirmation of the conclusions of the various authors is needed. The retina obtains its vitamin A from the blood. It is known that vitamin A may disappear from the blood during starvation and during infections, even though considerable stores may remain in the liver.[25] Consequently, further observations are necessary in order to establish the value of tests of adaptation in dim light as a criterion of the reserves of vitamin A in the body.

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[23] Isaacs, B. L.; Jung, F. T., and Ivy, A. C.: Vitamin A Deficiency and Dark Adaptation, J. A. M. A. **111:** 777 (Aug. 27) 1938.

[24a] Edmund, C., and Clemmesen, S.: On Deficiency of A Vitamin and Visual Dysaptation, Copenhagen, Levin and Munksgaard, 1936;

Medicaments, Am. J. Dis. Child. **53:** 89 (Jan., part 1) 1937; Studies on Hypovitaminosis A: III. Clinical Experiments in the Vitamin A Balance in Children After Various Diets, ibid. **53:** 1179 (May) 1937.

[25] Clausen, S. W.: Limits of the Anti-Infective Value of Provitamin A (Carotene), J. A. M. A. **101:** 1384 (Oct. 28) 1933.

Xerophthalmia and Keratomalacia. — These are symptoms of severe deficiency of vitamin A. Xerophthalmia, or dryness of the conjunctivae, is associated with atrophy of the para-ocular glands, with metaplasia of the epithelium of the glands and conjunctivae, and with loss of sensation in the conjunctiva, especially affecting the cornea. At first the dryness may be overlooked and may be associated with photophobia.[22c] Triangular white spots—Bitôt's spots—appear in the palpebral fissure; irregular wrinkled patches of xerosis are found over the bulbar conjunctivae and later over the cornea. A characteristic light brown pigmentation develops in the conjunctiva.[26] The superficial layers of the cornea degenerate. In the final stage, corneal softening, or keratomalacia, develops; severe impairment of vision results and permanent blindness is likely to follow.[27]

Xerophthalmia is rare in the United States. A few clinical and pathologic studies have been published.[28] The disease may appear in epidemic form during periods of famine or in persons receiving a deficient diet for some other reason. It has been observed in prisoners, slaves and workmen.[29] A very remarkable epidemic occurred during the World War in Denmark as a result of extensive exportation of dairy products and of the use of cheaper fats lacking in vitamin A for food.[30]

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[26] Mu, J. W.; Frazier, C. N., and Pillat, Arnold: Melanin Pigment of the Skin and Conjunctiva in Avitaminosis A in Man, Chinese J. Physiol. **11**: 247 (March 1) 1937.

[27] Mori, M.: Ueber den sogenannten Hikan (Xerosis conjunctivae infantum ev. Keratomalacie), Jahrb. f. Kinderh. **59**: 175, 1904. Mori, S.: The Changes in the Para-Ocular Glands Which Follow the Administration of Diets Low in Fat-Soluble A, with Notes of the Effect of the Same Diets on the Salivary Glands and the Mucosa of the Larynx and Trachea, Bull. Johns Hopkins Hosp. **33**: 357 (Oct.) 1922. Osborne, T. B., and Mendel, L. B.: Ophthalmia as a Symptom of Dietary Deficiency, Am. J. Physiol. **69**: 543 (Aug.) 1924. Bloch, C. E.: Blindness and Other Diseases in Children Arising from Deficient Nutrition (Lack of Fat-Soluble A Factor), Am. J. Dis. Child. **27**: 139 (Feb.) 1924. Pillat.[32]

[28] Little, W. S.: Kerato-malacie (Sphacelus) with a Case, Philadelphia M. Times **12**: 490 (April 22) 1882. Weeks, J. E.: Xerosis of the Conjunctiva in Infants and Children, Arch. Ophth. **15**: 332, 1886. Kollock, C. W.: A Form of Xerosis, Ophth. Rev. **9**: 249 (Sept.) 1890. Ross, S. G.: Nutritional Keratomalacia in Infants, with a Report of Four Cases, Am. J. Dis. Child. **22**: 232 (Sept.) 1921. Wilson, J. R., and DuBois, R. O.: Report of a Fatal Case of Keratomalacia in an Infant with Postmortem Examination, ibid. **26**: 431 (Nov.) 1923. Blackfan, K. D., and Wolbach, S. B.: Vitamin A Deficiency in Infants, J. Pediat. **3**: 679 (Nov.) 1933. Thorson, J. A.: Nutritional Xerophthalmia, J. A. M. A. **103**: 1438 (Nov. 10) 1934.

[29] Wright, R. E.: Keratomalacia in Southern India, Brit. J. Ophth. **6**: 164 (April) 1922. Mitchell, J. P.: Observations on Health in Relation to Diet in His Majesty's Central Prison, Uganda: I. Prison Diets and Morbidity, East African M. J. **10**: 38 (May) 1933. Loewenthal, L. J. A.: The Manifestations of Vitamin A Deficiency in Man, Ann. Trop. Med. **29**: 407 (Dec.) 1935.

The Treatment of Night Blindness Due to Vitamin A Deficiency.—Patients with normal power to absorb carotene and vitamin A may be treated by diet alone; the diet should include liberal amounts of milk, butter and green vegetables; cod liver oil or some other preparation of vitamin A or of carotene also should be administered. An improvement in dark adaptation may be demonstrable in a few minutes or hours after the ingestion of highly potent fish liver oils.[24] Jeghers [22c] reports that visual acuity was restored within two or three weeks in adults with night blindness who received a daily dose of carotene equivalent to 10,000 units of vitamin A. In certain rare cases, gastro-intestinal disease may result in faulty absorption, as in the patient of Wilbur and Eusterman,[31] whose diet had been adequate but who recovered from night blindness only after repair of a gastro-colic fistula.

SKIN

Dryness and scaliness of the skin, with a tendency to infection, were described in cases of xerophthalmia by Pillat.[32] Characteristic lesions of the human skin due to vitamin A deficiency were first recognized as such by Frazer and Ch'uan-K'uei [33] in Chinese soldiers. Later reports confirm their results and add a few facts.[34] The lesions usually occur in sexually mature persons between 16 and 30 years of age and not in infants. They are much more frequent in men than in women. Ninety per cent of the patients have obvious ocular manifestations of vitamin A deficiency. The lesions appear first on the anterolateral surface of the thighs and posterolateral portion of the upper forearms and later spread to adjacent areas of the skin. They consist of pigmented papules, up to 5 mm. in diameter, at the site of the hair follicles, from which project keratotic plugs. In about one third of the cases, pustules develop. The skin is not oily and does not sweat. Microscopic section shows atrophy of the pilosebaceous glands with metaplasia and hyperkeratinization of the epithelium. Secondary infection may occur but hemorrhage does not. The treatment consists of a diet including butter, eggs, liver and cod liver oil (as much as 30 cc. a day). The first sign of improvement is a return of moisture to the skin. The keratotic papules heal slowly after a few weeks.

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[30] Widmark, E.: Vitamin A Deficiency in Denmark and Its Results, Lancet **1:** 1206 (June 14) 1924. Bloch, C. E.: Effects of Deficiency in Vitamins in Infancy, Am. J. Dis. Child. **42:** 263 (Aug.) 1931. Blegvad.[1]

[31] Wilbur, D. L., and Eusterman, G. B.: Nutritional Night Blindness: Report of a Case, J. A. M. A. **102:** 364 (Feb. 3) 1934.

[32] Pillat, Arnold: Does Keratomalacia Exist in Adults? Arch. Ophth. **2:** 256 (Sept.), 399 (Oct.) 1929.

[33] Frazier, C. N., and Ch'uan-K'uei, Hu: Cutaneous Lesions Associated with a Deficiency of Vitamin A in Man, Arch. Int. Med. **48:** 507 (Sept.) 1931.

[34] Loewenthal, L. J. A.: A New Cutaneous Manifestation in the Syndrome of Vitamin A Deficiency, Arch. Dermat. & Syph. **28:** 700 (Nov.) 1933. Scheer, Max, and Keil, Harry: Follicular Lesions in Vitamin A and C Deficiencies, ibid. **30:** 177 (Aug.) 1934. Sweet, L. K., and K'ang, H. J.: Clinical and Anatomic Study of Avitaminosis A Among the Chinese, Am. J. Dis. Child. **50:** 699 (Sept.) 1935. Reiss, F.: A Contribution to Cutaneous Manifestations of Vitamin A Deficiency, Chinese M. J. **50:** 945 (July) 1936. Frazier, C. N., and Ch'uan-K'uei, Hu: Nature and Distribution According to Age of Cutaneous Manifestations of Vitamin A Deficiency: A Study of 207 Cases, Arch. Dermat. & Syph. **33:** 825 (May) 1936.

GASTRO-INTESTINAL TRACT

The Absorption of Vitamin A and of Carotene.—Drummond, Bell and Palmer[35] studied the absorption of vitamin A and carotene in a patient with a fistula of the thoracic duct opening into the pleural cavity. Analysis of the fluid from the chest, made after the administration of vitamin A or of carotene by mouth, showed that very little of the carotene had passed into the chylous fluid, whereas nearly all of the vitamin A could be recovered. The vitamin A of natural oils and fats occurs as an ester; in preparations made by saponification it occurs as a free alcohol. When the vitamin was administered as the free alcohol, it was found in the lymph mainly in the esterified condition. It therefore appears that the mechanism of absorption of vitamin A includes linkage with fatty acids, probably in the intestinal wall. Such linkage is impossible in the case of the hydrocarbon carotene, a fact that may explain its lower coefficient of absorption. There is evidently always some loss of vitamin A or of carotene in the stools; in diets low in vitamin A, a negative balance may be demonstrated.[36] Nothing is known about the degree of destruction of vitamin A or of carotene in the gastro-intestinal tract under normal or pathologic conditions. If liquid petrolatum is administered with the vitamin, considerable loss occurs.[37] The loss is not so great if the oil is administered separately, and the effect is much greater in the case of carotene than in the case of vitamin A itself.

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[35] Drummond, J. C.; Bell, M. E., and Palmer, E. T.: Observations on the Absorption of Carotene and Vitamin A, Brit. M. J. **1**: 1208 (June 15) 1935.

[36] Rowntree, J. I.: A Study of the Absorption and Retention of Vitamin A in Young Children, J. Nutrition **3**: 265 (Nov.) 1930.

Exclusion of bile from the alimentary tract interferes with absorption of carotene and possibly of vitamin A. Altschule[38] found microscopic evidence of vitamin A deficiency in six of eleven infants dying of congenital atresia of the bile ducts. In rats in which a fistula had been made between the gallbladder and the colon and which had been fed diets free of vitamin A, Greaves and Schmidt[39] found that carotene was not absorbed. If glycodeoxycholic acid or deoxycholic acid was administered with the carotene, it was absorbed. Vitamin A, as such, was however absorbed by the rat with the internal bile fistula.

During infections, both carotene and vitamin A are poorly absorbed.[40]

Patients with steatorrhea absorb vitamin A poorly.[41]

The faulty absorption of carotene from the gastrointestinal tract—and in some cases, of vitamin A—under the conditions just discussed explains the occurrence of symptoms of vitamin A deficiency in persons consuming adequate diets, and the occasional failure of therapy with vitamin A or carotene when given by mouth. In certain rare cases, it may be advisable to administer vitamin A or carotene by injection, as in a case observed by Bloch.[42]

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[37] Rowntree, J. I.: The Effect of the Use of Mineral Oil upon the Absorption of Vitamin A, J. Nutrition **3:** 345 (Jan.) 1931. Jackson, R. W.: The Effect of Mineral Oil Administration upon the Nutritional Economy of Fat-Soluble Vitamins: I. Studies with the Vitamin A of Butter Fat, ibid. **4:** 171 (July) 1931. Jackson, R. W.: The Effect of Mineral Oil Administration upon the Nutritional Economy of Fat-Soluble Vitamins: II. Studies with the Vitamin A Factor of Yellow Corn, ibid. **7:** 607 (June) 1934. Dutcher, R. A.; Harris, P. L.; Hartzler, E. R., and Guerrant, N. B.: Vitamin Studies: XIX. The Assimilation of Carotene and Vitamin A in the Presence of Mineral Oil, ibid. **8:** 269 (Sept.) 1934.

[38] Altschule, M. D.: Vitamin A Deficiency in Spite of Adequate Diet in Congenital Atresia of Bile Ducts and Jaundice, Arch. Path. **20:** *845 (Dec.) 1935.*

[39] Greaves, J. D., and Schmidt, C. L. A.: On the Absorption and Utilization of Carotene and Vitamin A in Choledochocolonostomized Vitamin A Deficient Rats, Am. J. Physiol. **111:** 492 (April) 1935.

[40] Clausen, S. W.: Limits of the Anti-Infective Value of Provitamin A (Carotene), J. A. M. A. **101:** 1384 (Oct. 28) 1933. Heymann, Walter: Absorption of Carotene, Am. J. Dis. Child. **51:** 273 (Feb.) 1936.

[41] Chesney, Jack, and McCoord, Augusta B.: Vitamin A of Serum Following Administration of Haliver Oil in Normal Children and in [illegible]

Vitamin A in Case of Celiac Disease: Studies on Hypovitaminosis A, Hospitalstid. **79:** 689 (July 7) 1936; abstr. J. A. M. A. **107:** 918 (Sept. 12) 1936. Bloch.[42]

Vitamin A in the Treatment of Gastro-Intestinal Disorders.—Manville[43] stated that the secretion of gastric mucus was decreased in rats fed diets deficient in vitamin A but that the secretion of acid was not impaired. The p_H of the gastric contents fell from normal values of 3.4 to 3.0-2.5 during avitaminosis. Gastric ulcers were found in from 60 to 100 per cent of the rats. The feeding of Fogelson's mucin did not relieve the ordinary symptoms of avitamosis but tended to cure the ulcers. In a later paper Manville[44] found that the number of mucus-secreting elements of the intestinal tract decreased, exposing the mucous membrane to injury from solid components of the food and feces. Occult blood in the stools was the earliest evidence of deficiency of vitamin A. Olcott[45] was unable to find ulceration of the gastro-intestinal tract in avitaminosis A.

Boller[46] has treated cases of hypo-acidity and of gastric and of duodenal ulcer with vitamin A, stating that increase in gastric acidity resulted and that symptoms and signs of ulcer diminished.

Obviously, the experimental basis for the use of vitamin A in gastro-intestinal disorders is not yet clearly established.

THYROID

In 1926 Abelin[47] observed that the toxic effects due to feeding thyroid gland to rats could be lessened by diets rich in cod liver oil or in egg yolk. He later published extensive studies[48] showing the beneficial effects of diets high in protein, fats and bone marrow, provided cod liver oil, egg yolk and dried powdered vegetables also were included in the diets. His purpose was to devise a diet for the treatment of hyperthyroidisms in man; he did not direct his studies to the discovery of any one particularly beneficial constituent. That step was taken by von Euler and Klussmann.[49] In their experiments, rats were fed diets in which the only source of vitamin A was known amounts of carotene. The loss in weight of the animals due to daily injections of thyroxine could be prevented by the administration of carotene. In the following year Abelin[50] showed that injections of thyroxine decrease the stores of carotene and vitamin A in the liver of guinea pigs consuming a diet very low in carotene and receiving intraperitoneal injections of colloidal carotene. According to Eufinger and Gottlieb,[51] the metamorphosis of tadpoles treated with thyroxine may be delayed by the administration of human plasma or of vitamin A. Fasold and Peters[52] repeated the experiments of von Euler and Klussmann and of Abelin, using a concentrate of fish liver oil ("Vogan"[53]) as the source of vitamin A instead of carotene. The dose was so large (1 cc., or 40,000 units of vitamin A daily) that symptoms of hypervitaminosis were produced. These symptoms could be prevented by administration of thyroxine and could be cured, if paralysis had not developed. These authors demonstrated that thyroxine does not prevent the conversion of carotene to vitamin A in the rat and that the beneficial results in hypervitaminosis are due to the rapid destruction of the vitamin. There is in fact evidence that the conversion of carotene to vitamin A may be deficient in hypothyroidism. The milk of goats consuming their normal diet is pure white; after thyroidectomy the milk becomes yellow. Fasold and Heidemann[54] showed that the yellow color of such milk is due to carotene and that vitamin A is no longer present in it. The liver of guinea pigs after thyroidectomy contains carotene but no vitamin A.[55] Wendt[56] reported that the blood plasma of persons with cretinism contains little vitamin A; after oral administration of carotene there is little rise in the vitamin A of the plasma. This, according to Wendt, is due to a failure of conversion of carotene to vitamin A in cretinism. In hyperthyroidism low values of vitamin A are also found; in this case, because of rapid destruction of vitamin A. After successful treatment with iodine or by surgery, the values increase. After a large dose of vitamin A, the rise in plasma vitamin A is less marked and is more transient than in normal persons.

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[42] Bloch, C. E.: Light and the A-Body, Am. J. Dis. Child. **31:** 315 (March) 1926.

[43] Manville, I. A.: The Production of Gastric Ulcers by Dietary Means, Am. J. Physiol. (Society Proc.) **105:** 70 (July) 1933.

[44] Manville, I. A.: The Interrelationship of Vitamin A and Glucuronic Acid in Mucine Metabolism, Science **85:** 44 (Jan. 8) 1937.

[45] Olcott, H. S.: Vitamin A Deficiency in the Dog, Proc. Soc. Exper. Biol. & Med. **30:** 767 (March) 1933.

[46] Boller, R.: Ueber die Behandlung verschiedener Magenkrankheiten mit Vitamin A (Vogan), Ztschr. f. klin. Med. **130:** 163, 1936.

[47] Abelin, I.; Goldener, E., and Kobori, B.: Ueber die Bedeutung des Fettes für die Stoffwechselwirkung der Schilddrüse, Biochem. Ztschr. **174:** 232, 1926.

[48] Abelin, I.: Ernährung und Schilddrüsenwirkung: I. Mitteilung. Einfluss des Caseins auf den hyperthyreotischen Stoffwechsel, Biochem. Ztschr. **228:** 165, 1930. Abelin, I.; Knuchel, M., and Spechtin, W.: Ernährung und Schilddrüsenwirkung: II. Ueber die Bedeutung der Vitamine für den Verlauf der experimentellen Hyperthyreose, ibid., p. 189. Abelin, I.: Ernährung und Schilddrüsenwirkung: III. Ueber den Einfluss bestimmt zusammengesetzter Nahrungsmischungen auf die hyperthyreotischen Stoffwechselstörungen, ibid., p. 211.

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[49] von Euler, H., and Klussmann, E.: Carotin (Vitamin A) und Thyroxin, Ztschr. f. physiol. Chem. **213**: 21, 1932; Vitamin A, und Follikulin, Arkiv. för Kemi, Mineralogi och Geologi, 1932, 11B, part 2, No. 1, pp. 1-6.

[50] Abelin, I.: Ueber die Beziehungen zwischen Carotin (Vitamin A) und Thyroxin, Ztschr. f. physiol. Chem. **217**: 109, 1933.

[51] Eufinger, H., and Gottlieb, J.: Wirkungskoeffizienten des Thyroxineffektes im biologischen Versuch, Klin. Wchnschr. **12**: 1397 (Sept. 9) 1933.

[52] Fasold, H., and Peters, H.: Ueber den Antagonismus zwischen Thyroxin und Vitamin A, Ztschr. f. d. ges. exper. Med. **92**: 57, 1933-1934.

[53] "Vogan" is a concentrate of fish liver oils; 1 cc. contains 40,000 rat units of vitamin A, an unstated amount of vitamin D, and about 6 micrograms of iodine.

The evidence just presented appears to indicate that an antagonism exists between the action of thyroxine and that of vitamin A. It must be pointed out, however, that relatively large doses of both substances were used; also that few animals were used in some of the experiments. It seems reasonable to conclude that vitamin A may be more rapidly destroyed by an organism in which the metabolic rate is increased from any cause, including hyperthyroidism. But it is not so easy to understand how vitamin A can decrease the activity of thyroxine. A few observations have been published to show that thyroxine is destroyed in vitro by carotene.

Treatment of Adolescent Goiter with Vitamin A.—Fasold[57] administered "Vogan" (30 drops, three times a day) to seven girls with adolescent goiter; none had signs of hypothyroidism or hyperthyroidism. After three or four weeks the struma in each case had regressed to a remarkable degree, but several weeks after the drug was discontinued the struma recurred.

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[54] Fasold, H., and Heidemann, E. R.: Ueber die Gelbfärbung der Milch thyreopriver Ziegen, Ztschr. f. d. ges. exper. Med. **92**: 53, 1933-1934.

[55] Schneider, E., and Widmann, E.: Die Hepatohormonale Steuerung des Vitamin-A-Umsatzes und die Aetiologie der Ostitis deformans Paget, Klin. Wchnschr. **14**: 1786 (Dec.) 1935.

[56] Wendt, H.: Ueber Veränderungen im Karotin-Vitamin-A-Haushalt beim Myxödem und bei Kretins, München. med. Wchnschr. **82**: 1679 (Oct.) 1935.

[57] Fasold, H.: Ueber die Wirkung des Vitamin A auf das Ovar des Puberstätsalters, Klin. Wchnschr. **16**: 90 (Jan. 16) 1937.

The effect cannot necessarily be ascribed to vitamin A, for "Vogan" in the dose mentioned would furnish about 3.6 micrograms of iodine a day.[58]

Treatment of Hyperthyroidism with Vitamin A.—Wendt [58] states that after large daily doses of "Vogan" (30 drops, three times a day, the total, 90 drops, contains about 3.6 micrograms of iodine and 145,000 "biologic units" vitamin A) three patients gained weight and their basal metabolic rate returned nearly to normal; two other patients improved. Dietrich [59] noted improvement in three of six patients (apparently permanent improvement in two) and none in two. The improvement required weeks. Abelin [60] regards the use of vitamin A as an aid in the treatment with iodine and with diiodotyrosine, stating that it greatly lessens the crises and the temporary exacerbations that are liable to occur with these forms of medication. Obviously, no conclusions as to the value of vitamin A in this disease are justified until more cases are reported and until the reports deal more critically with the other factors which may bring about improvement, especially the factors associated with the other constituents of the preparations used, with rest and with improved mental outlook.

URINARY SYSTEM

Urinary Lithiasis.—The assertion that diets deficient in vitamin A may cause urinary calculus and that the administration of vitamin A together with an acid ash diet may aid in the solution of urinary calculi has been recently reviewed by the Council on Pharmacy and Chemistry.[61] The Council concludes that there is no doubt that urinary stone may be produced in experimental animals by diets deficient in vitamins A and D and unbalanced in mineral content.[62] But the majority of investigators fail to find that a deficiency of vitamin A alone will cause stone. Higgins[63] is the chief proponent of the claim for the specific effect of lack of vitamin A in the production of urinary stone in man and of its solution by acid ash diets and vitamin A. Kayser, who discussed the paper of Higgins, pointed out that lithiasis, when it does occur in the rat with avitaminosis, is a very late manifestation. Children with deficiency of vitamin A rarely have calculi. In human beings with calculi, epithelial keratinization, the specific lesion of avitaminosis A, is lacking. In a large series of cases of stone, Kayser has failed to find evidence of dietary deficiency in a single case. Many of his patients had had diets rich in milk and eggs. In his experimental and clinical work, Higgins did not try separately the effects of administering vitamin A and acid-producing salts. Since the report was published, Oppenheimer and Pollack[64] have treated a series of cases of urinary stone with diets rich in vitamins A, B, D, E and G and having an acid ash, administering sufficient ammonium chloride to assure an acid reaction in the urine (p_H below 5.2). Fifty-two patients with stone were studied; twenty-seven were observed for from six to sixteen months (on the average eleven months). In two, uremia and acidosis seemed to have been precipitated by the regimen. In none of the subjects was even partial solution of the calculi demonstrable by x-ray examination; in five, the size of the stone increased and in one, a new stone formed during the treatment.

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[58] Wendt, H.: Ueber die Behandlung der Basedow'schen Krankheit mit grossen Dosen Vitamin A (Vogan), München. med. Wchnschr. **82**: 1160 (July) 1935.

[59] Dietrich, H. E.: Vitaminbehandlung der Schilddrüsenüberfunktion mit Vogan, München. med. Wchnschr. **83**: 313 (Feb.) 1936.

[60] Abelin, I.: Ueber die medikamentöse Anwendung des A-Vitamins bei der Hyperthyreose, Schweiz. med. Wchnschr. **66**: 1106 (Nov.) 1936.

[61] Vitamin A and Urinary Lithiasis, Reports of the Council on Pharmacy and Chemistry, J. A. M. A. **105**: 1983 (Dec. 14) 1935.

[62] McCarrison, Robert: The Experimental Production of Stone in the Bladder, with a Note on Pernicious Anemia and Epidemic Dropsy, Indian J. M. Research **14**: 895 (April) 1927; The Experimental Production of Stone in the Bladder, Brit. M. J. **1**: 717 (April 16) 19 van Leersum, E. C.: Vitamin A Deficiency and Urolithiasis, J. B. Chem. **76**: 137 (Jan.) 1928.

It may be concluded that, although urinary calculi may be produced in experimental animals by feeding special diets, the factors involved are not yet clearly defined. Further study is required before the results can be applied to man. The view that diets deficient in vitamin A cause urinary lithiasis in man is not supported by the clinical evidence.

REPRODUCTIVE SYSTEM

In the Male.—It has been shown that the testes of rats degenerate in the course of deficiency of vitamin A. Evans[65] has demonstrated that males eating a diet free of vitamin A but adequate in vitamin E become sterile in three months, whereas those receiving adequate vitamin A but no vitamin E do not become sterile until later. The effects of moderate deficiency of vitamin A on fertility in man are unknown, nor is anything known about the relative importance of moderate deficiency in men and in women.

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[63] Higgins, C. C.: Production and Solution of Urinary Calculi, J. A. M. A. **104:** 1296 (April 13) 1935.

[64] Oppenheimer, G. D., and Pollack, Herbert: Attempted Solution of Renal Calculi by Dietetic Measures, J. A. M. A. **108:** 349 (Jan. 30) 1937.

[65] Evans, H. M.: Testicular Degeneration Due to Inadequate Vitamin A in Cases Where Vitamin E Is Adequate, Am. J. Physiol. **99:** 477 (Jan.) 1932.

In the Female.—The vaginal mucous membrane becomes permanently cornified in animals deprived of vitamin A. Observation of the vaginal smear may be used as a method of detecting vitamin A deficiency.[66] In castrated female rats in which vitamin A deficiency has been produced, injections of the principle from human placenta fail to cause the appearance of mucoid cells in the vagina;[67] and in castrated female mice with diets deficient in carotene and vitamin A, estrus can be produced by injections of estrogen together with the administration of carotene, but not by estrogen or by the carotene alone.[68] In uncastrated rats, Mason and Ellison,[69] by the use of the lavage method of obtaining smears stained supravitally with neutral red, have been able to demonstrate the presence of estrous cycles even in A-deficient animals in which abnormal cornification has become too severe to permit of their identification by dry stained smears. In castrated animals, small numbers of cornified cells continue to appear even after vitamin A therapy. It is therefore evident that the influence of deficiency of vitamin A on the estrous cycle is not only directly on the vaginal mucous membrane but on or through the ovary also. The effect on the hypophysis is as yet unknown. The influence of excessive doses of vitamin A (as carotene) on the sexual functions of female rats has been studied by Sherwood, Brend and Roper.[70] In rats receiving a daily dose of carotene equivalent to from

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66. Baumann, C. A., and Steenbock, Harry: Vaginal Smear Method of Determining Vitamin A, Science **76:** 417 (Nov. 4) 1932. Hohlweg, Walter, and Dohrn, Max: The Determination of Vitamin A by Means of Its Influence on the Vaginal Contents of the Rat, Biochem. J. **30:** 932 (May) 1936.

67. Aberle, S. B. D.: The Interrelation of a Gonadotropic Hormone and Vitamin A, Am. J. Physiol. **106:** 267 (Nov.) 1933.

68. von Euler, H.; Zondek, Bernhard, and Klussmann, E.: Carotinoide, Vitamin E, und Sexualhormone, Arkiv. för Kemi, Mineralogi och Geol., 1932, 11B, No. 2, pp. 1-5.

69. Mason, K. E., and Ellison, E. T.: Changes in the Vaginal Epithelium of the Rat After Vitamin A Deficiency, J. Nutrition **9:** 735 (June) 1935; Demonstration of Oestrus in the Vitamin A Deficient Rat by Supravital Study of the Vaginal Smears, ibid. **10:** 1 (July) 1935.

70. Sherwood, T. C.; Brend, M. A., and Roper, E. A.: Changes in the Vaginal Epithelium of the Rat on an Excessive Vitamin A Diet, J. Nutrition **11:** 593 (June) 1936.

1,500 to 3,750 international units of vitamin A per day, the vaginal smears did not progress from the stage of nucleated epithelium to that of cornified cells. Normal estrous cycles did not return for twenty days after the feeding of the carotene had been discontinued. During the period of ingestion of the carotene, the rats showed no desire to copulate.

In Human Subjects.—Simpson and Mason [71] report that the administration of cod liver oil or of halibut liver oil relieved symptoms and pathologic signs in thirty cases of senile vaginitis. Fasold [57] administered a concentrate of fish liver oil ("Vogan"; 30 drops, three times a day = 145,000 international units of vitamin A) to seven adolescent girls with large goiters of puberty. In four of the girls the menses ceased for the period of seven months during which vitamin A was administered and were resumed from four to six weeks after the treatment was discontinued. Menstrual disturbances that had been complained of before the treatment did not reappear.

THE BLOOD AND BLOOD FORMING ORGANS

Anemia and Vitamin A Deficiency. — French authors [72] report that regeneration of hemoglobin and red cells in the anemia induced in dogs by repeated bleedings is facilitated by the administration of carotene. This work lacks confirmation. In seven patients among thirty-five with keratomalacia, Pillat and Yang [73] found an anemia which they believe could be explained only as a result of deficiency of vitamin A and which was cured rapidly with cod liver oil. The belief that pernicious anemia is associated with deficiency of vitamin A in the diet has now been discredited. It seems clear that anemia is not a regular symptom of deficiency of vitamin A, and the use of vitamin A for the treatment of the anemias does not rest on a secure foundation.

The Thrombocytes and Vitamin A.—The assertion of Cramer, Drew and Mottram [74] that the number of thrombocytes is diminished in avitaminosis A seems to have been disproved by Bedson and Zilva,[75] who used more accurate methods of counting the blood platelets, and by Falconer and Peachey.[76] Their control animals, however, received a perfectly normal diet and not the basal diet plus vitamin A. Lorenz[77] believed that "Vogan," a concentrate of fish liver oil, enables the body to "regulate" the number of thrombocytes. In children and in guinea pigs he observed that, following the administration of the drug, originally low platelet counts rose, originally high counts fell. He attributes this effect to vitamin A. Schiff and Hirschberger[78] at first agreed but later stated that the solvent in "Vogan," sesame oil, contains a hitherto undescribed substance which they designate as vitamin T, which causes a rise in blood platelets.

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[71] Simpson, J. W., and Mason, K. E.: A New Concept of Senile Vaginitis, Am. J. Obst. & Gynec. **32**: 125 (July) 1936.

[72] Binet, L., and Strumza, M. V.: Le carotène, son pouvoir hématopoiétique, Presse méd. **40**: 41 (Jan. 9) 1932.

[73] Pillat, Arnold, and Yang, C. S.: The Blood Picture in Keratomalacia of Adults, Arch. Ophth. **4**: 309 (Sept.) 1930.

[74] Cramer, W.; Drew, A. H., and Mottram, J. C.: On the Function of the Lymphocyte and of Lymphoid Tissue in Nutrition, with Special Reference to the Vitamin Problem, Lancet **2**: 1202 (Dec. 10) 1921.

The Granulocytes and Vitamin A.—Crimm and Short[79] state that the earliest manifestation of cellular changes associated with vitamin A deficiency in dogs and rats is a "neutrophil index lag"; large doses of vitamin A administered to normal animals or to normal persons (70,000 international units daily) cause a shift to the left in the neutrophilic index. This shift is not due to vitamin D. It would seem evident from this work that the lag is not marked enough to be of value in the diagnosis of avitaminosis; the shift to the left requires large amounts of vitamin A administered over a long period and is not present in active infections, possibly because a shift has already occurred.

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[75] Bedson, S. P., and Zilva, S. S.: The Influence of Vitamin A on the Blood Platelets of the Rat, Brit. J. Exper. Path. **4:** 5 (Feb.) 1923.

[76] Falconer, E. H., and Peachey, G.: Blood Counts in Vitamin A Deficiency Disease, with Special Reference to the Platelets, Am. J. Physiol. **76:** 145 (March) 1926.

[77] Lorenz, E.: Der Einfluss des A-Vitamins auf die Thrombocyten, Ztschr. f. Kinderh. **58:** 504 (Dec.) 1936.

[78] Schiff, E., and Hirschberger, C.: Weitere Untersuchungen zur Frage des Morbus maculosus Werlhofi: II. Einfluss der Vitamine A, B_1, B_2, C, D und des Eigelbs auf die Zahl der Thrombozyten bei gesunden Kindern Jahrb. f. Kinderh. **146:** 181, 1935; Weitere Untersuchungen zur Frage des Morbus maculosus Werlhofi: III. Einfluss der Vitamine C, A, B_2 und des Eigelbs auf die Zahl der Thrombozyten und die Blutungszeit beim Morbus mac. Werlhofi, ibid., p. 191; Weitere Untersuchungen zur Frage des Morbus maculosus Werlhofi: IV. Ein bisher unbekannter Thrombozytose herbeiführender Stoff, der fettlösliche T-Faktor, ibid., p. 293; Weitere Untersuchungen zur Frage des Morbus maculosus Werlhofi: V. A-Vitamin und Thrombozytose, ibid. **147:** 81, 1936.

[79] Crimm, P. D., and Short, D. M.: Qualitative Blood Cell Changes in the Rat Due to Vitamin A, Am. J. Physiol. **111:** 397 (March) 1935; Qualitative Blood Cell Changes in the Human Due to Vitamin A, J. Indiana M. A. **28:** 175 (April) 1935; Vitamin A Deficiency in the Dog, Am. J. Physiol. **118:** 477 (March) 1937

It may be concluded that no very striking effect of vitamin A on the cellular elements of the blood has been discovered.

VITAMIN A AND THE NERVOUS SYSTEM

Edward Mellanby [80] stated that, when diets containing a large amount of cereal (other than yellow maize) and deficient in vitamin A are eaten by young puppies, degenerative changes occur in the spinal cord; demyelinization can be demonstrated by the Marchi stain. The presence of any source of vitamin A in the diet (cod liver oil, butter or carotene) prevents or diminishes this change. He believed that the nervous symptoms in convulsive ergotism, pellagra, lathyrism, the subacute combined degeneration in pernicious anemia, tabes dorsalis and multiple sclerosis may be related to deficiency of vitamin A. In 1935 Mellanby [81] reported demyelinization, chiefly of the peripheral nervous system, in growing rabbits consuming a diet poor in vitamin A and reiterated his belief that diets high in cereal aggravate the condition. Later he ascribed the metaplasia of specific epithelium to lesions of the trophic nerve fibers due to deficiency of vitamin A.

Using adult dogs, Suzman, Muller and Ungley [82] were unable to produce lesions in the nervous system or abnormal nervous symptoms as a result of the ingestion of diets free of vitamin A and containing an excess of cereal. Their animals, at least toward the end of the experiment, had exhausted their stores of vitamin A. In a certain number of rats, but not in all, consuming diets deficient in vitamin A, Zimmerman [83] and Zimmerman and Cowgill [84] were able to observe incoordination and muscular weakness. In these rats only they observed degeneration of the spinal cord and of the peripheral nerves. The diets contained no cereal but contained an adequate amount of unsaturated fatty acids. Sutton, Setterfield and Krauss[85] were able to demonstrate in frozen sections with polarized light myelin degeneration of the peripheral nerves in rats receiving a diet deficient in vitamin A. The degeneration occurred at about the same time as did xerophthalmia. Aberle[86] found that the appearance of nervous symptoms was always later than the continuous appearance of cornified vaginal cells.

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[80] Mellanby, Edward: The Experimental Production and Prevention of Degeneration in the Spinal Cord, Brain **54**: 247 (Sept.) 1931.

[81] Mellanby, Edward: Lesions of the Central and Peripheral Nervous Systems Produced in Young Rabbits by Vitamin A Deficiency and a High Cereal Intake, Brain **58**: 141 (June) 1935.

[82] Suzman, M. M.; Muller, G. L., and Ungley, C. C.: An Attempt to Produce Spinal Cord Degeneration in Dogs Fed a High Cereal Diet Deficient in Vitamin A: The Incidental Development of a Syndrome of Anemia, Skin Lesions, Anorexia and Changes in the Concentration of Blood Lipoids, Am. J. Physiol. **101**: 529 (Aug.) 1932.

[83] Zimmerman, H. M.: Lesions of the Nervous System in Vitamin Deficiency: I. Rats on a Diet Low in Vitamin A, J. Exper. Med. **57**: 215 (Feb. 1) 1933.

[84] Zimmerman, H. M., and Cowgill, G. R.: Lesions of the Nervous System in Vitamin Deficiency: IV. The Effect of Carotene in the Treatment of the Nervous Disorder in Rats Fed a Diet Low in Vitamin A, J. Nutrition **11**: 411 (May) 1936.

Bartz and Schmitt[87] were able to demonstrate by satisfactory methods the presence of relatively large amounts of carotene and of vitamin A in the peripheral nerves of frogs and to show that starvation depletes the supply whereas feeding with carotene restores both the carotene and the vitamin A content.

More knowledge is obviously required as to (*a*) the function of vitamin A in the nervous system, (*b*) the other deficiencies in the diets used, (*c*) the influence of the age of the subject, and (*d*) the possible specific effects of cereals, before any intelligent application can be made of this experimental work to the treatment of nervous disorders in man.

TUMORS

The incorrect assumption that vitamin A is the "growth vitamin" has led several investigators to study the effect of vitamin A on the growth of tumors. The conflicting results were summarized by Baker.[88] She was able to show that highly purified vitamin A—but not carotene—in suitable concentration stimulates the growth of fibroblasts in tissue culture; higher concentrations are distinctly toxic. Kuh[89] reported that the amount of vitamin A present in the usual diet has no effect on the growth of transplanted tumors in mice but that large doses of carotene inhibit the growth of such tumors, provided the animal can convert the carotene to vitamin A. The effective dose on the basis of weight would be equivalent to 0.6 Gm. of carotene, i. e., 1,000,000 international units daily for a person weighing 60 Kg. Dittmar[90] reports that large doses of vitamin A decrease the percentage of successful tumor implants in mice. As the doses were from 1,000 to 2,000 rat units daily, it seems possible that he may have been dealing with a toxic effect due either to the vitamin itself or to some other substance in the concentrate. Lustig and Wachtel[91] believe that local applications of concentrates of vitamin A assist in the healing of malignant ulcers, provided radium also is used. The influence of vitamin A deficiency on the growth of tumors has been little studied, because complete deficiency is likely to be fatal before the growth of the tumors can be accurately observed. Wolbach and Howe[92] state their belief that in man defective formation of the enamel of the teeth, denticles, pulp bone and cemento-exostoses may reasonably be regarded as possibly due to vitamin A deficiency. Orten, Burn and Smith[93] produced moderate deficiency of vitamin A in rats by feeding the animals on a diet deficient in the vitamin from weaning until signs of deficiency developed, then supplementing the diet with from 0.7 to 6 international units of vitamin A daily. In those rats which survived for a year, they found quite constantly large painless odontomas of the maxillae developing from the embryonic cells of the pulp.

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[85] Sutton, T. S.; Setterfield, H. E., and Krauss, W. E.: Nerve Degeneration Associated with Avitaminosis A in the White Rat, Bull. 545, Ohio Agric. Exper. Sta., December 1934.

[86] Aberle, S. B. D.: Neurological Disturbances in Rats Reared on Diets Deficient in Vitamin A, J. Nutrition **7**: 445 (April) 1934.

[87] Bartz, J. P., and Schmitt, F. O.: Carotene and Associated Pigments in Medullated Nerve, Am. J. Physiol. **117**: 280 (Oct.) 1936.

[88] Baker, Lillian E.: Effect of Vitamin A on Proliferation of Fibroblasts, Proc. Soc. Exper. Biol. & Med. **33**: 124 (Oct.) 1935.

[89] Kuh, Clifford: A Study of Vitamin A in Relation to Experimental Cancer, Yale J. Biol. & Med. **5**: 123 (Dec.) 1932.

LOCAL TREATMENT OF BURNS, ULCERS AND INFECTIONS

The local treatment of infections, burns and fresh wounds with applications of ointments containing cod liver oil or concentrates has been enthusiastically advocated by Löhr.[94] He ascribed his apparently successful results in part to a bactericidal influence of the preparations used, in part to a stimulation of growth of epithelium. He presented illustrative cases but no controlled observations. He later [95] described the treatment of acute and chronic osteomyelitis by means of local applications of cod liver oil ointment and plaster bandages.

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[90] Dittmar, C.: Ueber den Einfluss der Kost auf das Wachstum von Impftumoren, Ztschr. f. Krebsforsch. **44:** 73, 1936.

[91] Lustig, B., and Wachtel, H.: Therapeutische Versuche mit Vitamin A beim Krebs, Ztschr. f. Krebsforsch. **44:** 53 (June) 1936.

[92] Wolbach, S. B., and Howe, P. R.: The Incisor Teeth of Albino Rats and Guinea Pigs in Vitamin A Deficiency and Repair, Am. J. Path. **9:** 275 (May) 1933.

[93] Orten, A. U.; Burn, C. G., and Smith, A. H.: Effects of Prolonged Chronic Vitamin A Deficiency in the Rat, with Special Reference to Odontomas, Proc. Soc. Exper. Biol. & Med. **36:** 82 (Feb.) 1937.

Andernach and Löhr [96] advocate the injection of cod liver oil into the joint spaces in chronic arthritis of various types; a rather severe (sterile?) inflammation ensues, following which improvement occurs, probably as a result of hyperemia. Their cases, however, are not analyzed from a statistical standpoint. Several other authors have reported good results from the use of local applications of ointments containing vitamins A and D.[97] Solutions of carotene in oil have been recommended by Rachet and Busson [98] for the local treatment of ulcerative colitis. The work was not controlled by the use of oil without carotene. Balachovski [99] used colloidal carotene in salt solution for the local treatment of superficial lesions of the eye, gingivitis, chilblains, fissures and inflammations. As he states that the treatment was just as effective after the solutions had become bleached, it is not possible to attribute his results to the action of carotene.

The clinical work just reviewed seems to have been done without a preliminary experimental study of the possible factors involved. The experimental work done by other investigators throws doubt on any specific action of the vitamins contained in the preparations applied locally. Lauber and Rocholl[100] find that the base of the ointment used is effective. Lundh[101] studied the healing of wounds produced in guinea pigs, treating the wounds on one side with various ointments and permitting the wound on the other side to heal without an ointment. Cod liver oil did not promote healing; the wounds treated with yellow petrolatum healed more slowly than the wounds exposed to dry air; the wounds treated by an ointment containing 10 per cent zinc oxide or 10 per cent zinc oxide and 1 per cent halibut liver oil healed in twenty-five days. Those treated with an ointment containing 10 per cent or 25 per cent halibut liver oil healed in twenty-three days. As he gives only the average time of healing and not the times of healing for the individual animals, it is not possible to accept his conclusion that the difference is significant, much less that it is a specific effect. Finally, the differences are so small that they by no means justify the enthusiasm of the clinical reports.

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[94] Löhr, W.: Ueber die Lebertransalbenbehandlung (mit und ohne Gipsverband) bei frischen Verletzungen, Verbrennungen, und phlegmonösen Entzündungen, Zentralbl. f. Chir. **61:** 1686 (July 21) 1934.

[95] Löhr, W.: Die Behandlung der akuten and chronischen Osteomyelitis der Röhrenknochen mit dem Lebertrangips, Deutsche med. Wchnschr. **62:** 997 (June 19) 1936.

[96] Andernach, F., and Löhr, W.: Die Behandlung der chronischen Arthritis mit Lebertran injektionen ins Gelenk, Zentralbl. f. Chir. **63:** 2493 (Oct. 17) 1936.

[97] Horn, Z., and Sándor, S.: Die lokale Anwendung des A-Vitamins in der Wundbehandlung, Deutsche med. Wchnschr. **60:** 1018 (July 6) 1934. [illegible], L.: [illegible] in der Wundbehandlung, Zentralbl. f. Chir. **62:** 3031 (Dec. 21) 1935. Sándor, S.: Vitamin A in the Local Treatment of Wounds, Lancet **2:** 738 (Sept. 26) 1936.

[98] Rachet, A. J., and Busson, A.: Le traitement des recto-colites ulcéreuses par la vitamine A, Paris méd. **95:** 308 (April 6) 1935.

[99] Balachovski, S.: Sur la possibilité d'insuffisances locales en vitamines, considérations théoriques et application thérapeutique, Presse méd. **42:** 1404 (Sept. 8) 1934.

VITAMIN A AND INFECTIONS

Severe spontaneous infections in animals with xerophthalmia were first reported by McCollum.[102] Such infections cannot be prevented by vitamin D but can be prevented by the administration of cod liver oil or of carotene in amounts sufficient to prevent the development of symptoms of deficiency of vitamin A.[103] Animals suffering from deficiency of vitamin A are more susceptible than control animals to a variety of infections artificially produced either by inoculation through the mouth or by intraperitoneal injection of cultures.[104] The loss of resistance may appear even before other symptoms of vitamin A deficiency,[105] and it may appear in animals receiving a diet considered nearly adequate, as in the experimental epidemics in mice studied by Webster and Pritchett[106] and by Pritchett.[107] The study of experimental epidemics, of obvious importance for infections in man, has demonstrated the possibility that moderate degrees of deficiency may lower resistance but that many more factors are involved than deficiency of vitamin A.[108]

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[100] Lauber, H. J., and Rocholl, H.: Experimentelle Untersuchungen über die Einwirkung Vitaminhaltiger Salben auf die Wundheilung, Klin. Wchnschr. **14:** 1143 (Aug. 10) 1935.

[101] Lundh, G.: Experimentelle Untersuchungen der Wundheilung bei Tieren mit Lebertran, Zentralbl. f. Chir. **63:** 2860 (Nov. 28) 1936.

[102] McCollum, E. V.: The Supplementary Dietary Relationships Among Our Natural Foodstuffs, J. A. M. A. **68:** 1379 (May 12) 1917. Drummond, J. C.: Researches on the Fat-Soluble Accessory Substance: I. Observations upon Its Nature and Properties, Biochem. J. **13:** 81 (May) 1919. Mellanby, Edward: An Experimental Investigation on Rickets, Lancet **1:** 407 (March 15) 1919. Daniels, Amy L.; Armstrong, Margaret E., and Hutton, Mary K.: Nasal Sinusitis Produced by Diets Deficient in Fat-Soluble A Vitamin, J. A. M. A. **81:** 828 (Sept. 8) 1923.

[103] Green, H. N., and Mellanby, Edward: Vitamin A as an Anti-Infective Agent, Brit. M. J. **2:** 691 (Oct. 20) 1928; Carotene and Vitamin A: The Anti-Infective Action of Carotene, Brit. J. Exper. Path. **11:** 81 (April) 1930.

The mechanism of lowered resistance to infection in vitamin A deficiency is not understood. Although poor general condition plays a role,[109] infection seems more likely to occur in animals suffering from a deficiency of vitamin A than in those with a deficiency of other vitamins. The characteristic lesion in animals with fully developed vitamin A deficiency is degeneration of specialized epithelium which is replaced by keratinizing epithelium. In the case of the respiratory tract, alterations of the mucous membranes are undoubtedly the chief cause of spontaneous infections. In case of infections following inoculation into the blood stream or peritoneal cavity, and in case of loss of resistance in moderate deficiency of vitamin A, some other mechanism must be involved. The majority of writers agree that both the normal antibodies of the serum and the power to produce antibodies are not affected by dietary deficiencies excepting in the terminal stages and that deficient animals are not more susceptible than controls to bacterial toxins.[110] The defensive response of the tissues in vitamin deficiency has not been adequately studied.

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[104] Ørskov, J., and Moltke, O.: Studien über den Infektionsmekanismus bei verschiedenen Paratyphus-Infektionen an weissen Mäusen, Ztschr. f. Immunitätsforsch. u. exper. Therap. **59:** 357 (Dec.) 1928. Ørskov, J., and Lassen, H. C. A.: Die Bedeutung der Grösse der primären Infektionsdosis bei einigen natürlichen Infektionen, Ztschr. f. Immunitätsforsch. u. exper. Therap. **67:** 137 (June) 1930. Lassen, H. C. A.: Vitamin A Deficiency and Resistance Against a Specific Infection, J. Hyg. **30:** 300 (Aug.) 1930; Experimental Studies on the Course of Paratyphoid Infections in Avitaminctic Rats, with Special Reference to Vitamin A Deficiency, Copenhagen, Levin and Munksgaard, 1931; Die Bedeutung der Vitamine für den Verlauf von Infektionen, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73:** 221 (Jan.) 1931.

[105] Boynton, L. C., and Bradford, W. L.: Effect of Vitamins A and D on Resistance to Infection, J. Nutrition **4:** 323 (Sept.) 1931.

[106] Webster, L. T., and Pritchett, I. W.: Microbic Virulence and Host Susceptibility in Paratyphoid Enteritidis Infection of White Mice: V. The Effect of Diet on Host Resistance, J. Exper. Med. **40:** 397 (Sept.) 1924.

[107] Pritchett, I. W.: Microbic Virulence and Host Susceptibility in Parathyphoid Enteritidis Infection of White Mice: XII. Effect of Diet on Host Resistance, Further Studies, J. Exper. Med. **46:** 557 (Oct.) 1927.

[108] Webster, L. T.: The Role of Microbic Virulence, Dosage and Host Resistance in Determining the Spread of Bacterial Infections Among Mice: I. Pasteurella Lepiseptica and Pasteurella Aviseptica Infections, J. Exper. Med. **52:** 901 (Dec.) 1930. Topley, W. W. C.; Greenwood, M., and Wilson, J.: The Effect of Diet in Epidemic Infections in Mice, J. Path. & Bact. **34:** 163, 1931. Watson, Marion: Studies on the Influence of Diet on Resistance to Infection: I. The Effect of Various Diets on the Fertility, Growth and Survival of Mice, J. Hyg. **37:** 396 (July) 1937; Studies on the Influence of Diet on Resistance to Infection: II. The Effect of Various Diets on the Resistance of Mice to Bacterial Infection, ibid. **37:** 420 (July) 1937.

[109] Arons, P., and van der Rijst, M. P. J.: The Cause of Infections Following Lack of Vitamin A, Nederl. tijdschr. v. geneesk. **76:** 5445 (Nov. 26) 1932. Harris, L. J.; Innes, J. R. M., and Griffith, A. S.: On the Pathogenesis of Avitaminosis A, Vitamin A as the Antikeratinizing Factor, Lancet **2:** 614 (Sept. 17) 1932.

Resistance to infection in man may be decreased in cases with marked deficiency of vitamin A. Bloch[111] reported that children suffering with xerophthalmia are liable to severe infections and that, even after recovery from the deficiency, their liability to death from infection remains high for years.[112] The majority of observers agree with Bloch's conclusions that infections are frequent and severe in cases of xerophthalmia, but Spence[113] failed to observe this. Moderate degrees of vitamin A deficiency have been assumed to lower resistance to infection in man. Four methods have been used to show that vitamin A deficiency might exist in the group subject to infection and not in the control group: (1) calculation of the intake of vitamin A in the two groups, (2) analysis of the liver for vitamin A in persons dying of infection and in those dying from accident, (3) analysis of the blood for carotene and for vitamin A, and (4) the demonstration of night blindness in the group subject to severe injection. The first method is essentially that of Barenberg and Lewis,[114] of Hess, Lewis and Barenberg[115] and of

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[110] Robertson, E. C.: The Vitamins and Resistance to Infection, Medicine **13**: 123 (May) 1934. Gellhorn, Ernst, and Dunn, Joan O.: The Influence of Lack of Vitamin A in the Diet on Phagocytosis Promoting Properties of the Blood Serum, J. Nutrition **13**: [illegible] (March) 1937. Torrance, C. C.: The Relation Between Vitamin A Metabolism and Susceptibility to Diphtheria Toxin, Am. J. Hyg. **18**: 375 (Sept.) 1933; Further Studies on the Relation Between Vitamin A Metabolism and Susceptibility to Bacterial Toxins, ibid. **23**: 74 (Jan.) 1936. Clausen.[117]

[111] Bloch, C. E.: Decline in Immunity as a Symptom Due to Deficiency in A Vitamin and in C Vitamin, Acta pædiat., Supp. 2, (Lecture, 14th Scandinavian Paediatric Congress) **7**: 61, 1928.

[112] Bloch, C. E.: Effects of Deficiency in Vitamins in Infancy, Am. J. Dis. Child. **42**: 263 (Aug.) 1931.

[113] Spence, J. C.: A Clinical Study of Nutritional Xerophthalmia and Night-Blindness, Acta pædiat. (Trans. Second Int'l Ped. Congress, Stockholm, Sweden) **11**: 541, 1930.

[114] Barenberg, L. H., and Lewis, J. M.: Relationship of Vitamin A to Respiratory Infection in Infants, J. A. M. A. **98**: 199 (Jan. 16). 1932.

[115] Hess, A. F.; Lewis, J. M., and Barenberg, L. H.: Does Our Dietary Require Vitamin A Supplement? J. A. M. A. **101**: 657 (Aug. 26) 1933.

Wright. Frosst, Richel and Lawrence.[116] They were unable to lessen the incidence or the severity of respiratory infection in groups of infants by administering cod liver oil, halibut liver oil or carotene. However, the infants in their control group were already receiving adequate amounts of vitamin A. It is also possible that an inadequate diet at an age earlier than that of their subjects may be of importance.[117] Analyses of the liver have been carried out by several authors,[118] who agree that low values are usual in the livers of persons dying of infections. But it must be emphasized that the stores of vitamin A are depleted during periods of underfeeding and by infection itself. Analyses of the blood for carotene and for vitamin A [119] also show a decrease during infection. In children subject to repeated respiratory infections, but not recently ill, the average level of blood carotene is lower than in other children. But the increased liability to infection which could be ascribed to low blood carotene is only about 10 per cent.[120] The recent rather intensive study of night blindness has afforded a more direct method of ascertaining the presence of moderate degrees of vitamin A deficiency. Jeghers [121] in a group of 162 medical students found fifty, or 36 per cent, whose visual adaptation to dim light was subnormal and fifty whom he regarded as certainly normal. The calculated daily intake of vitamin A in the subnormal group was 2,445 international units; in the normal group it was 5,500. The incidence of colds during the preceding year was nearly equal (2.2 per person vs. 2.1), but the duration was 7.4 days in the subnormal group and 5.4 days in the normal group. It is obvious that the differences are not very great and certainly do not justify terming vitamin A "the anti-infective vitamin." More studies of this type are essential.

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[116] Wright, H. P.; Frosst, J. B.; Richel, F., and Lawrence, M. R.: Vitamin A and the Common Cold, Canad. M. A. J. **25:** 412 (Oct.) 1931.

[117] Clausen, S. W.: The Influence of Nutrition upon Resistance to Infection, Physiol. Rev. **14:** 309 (July) 1934.

[118] Laqueur, E.; Wolff, L. K., and Dingemanse, E.: Ueber den Gehalt der Leber an Vitamin A (Im besonderen beim Menschen), Deutsche med. Wchnschr. **54:** 1495 (Sept. 7) 1928. Moore, T.: Vitamin A Reserves of the Human Liver in Health and Disease, with Special Reference to the Scope of Vitamin A as an Anti-Infective Agent, Lancet **2:** 669 (Sept. 24) 1932. Wolff, L. K.: On the Quantity of Vitamin A Present in the Human Liver, Lancet **2:** 617 (Sept. 17) 1932.

[119] McCoord, A. B., and Clausen, S. W.: Unpublished data.

[120] Clausen (footnotes 25 and 127).

[121] Jeghers, Harold: The Degree and Prevalence of Vitamin A Deficiency in Adults, with a Note on Its Experimental Production in Human Beings, J. A. M. A. **109:** 756 (Sept. 4) 1937.

Within the past few years, several authors have studied the effect of administering vitamin A on the incidence or on the course of infections. In these studies, no attempt was made to ascertain the presence or the absence of vitamin A deficiency. A favorable effect from administration of vitamin A has been claimed on the outcome in pneumonia by Donaldson and Tasker [122] and in measles by Ellison.[123] In studies which seem to have been better controlled, no favorable effect was found in pneumonia by Orenstein,[124] in measles by Mackay and her co-workers,[125] in the complications of scarlet fever by Sutliff and his co-workers,[126] or by Clausen,[127] and in pertussis by Sehestedt.[128] It was shown that the vitamin was absorbed.[127] The literature on the effect of the administration of vitamin A on the prophylaxis of illness, especially of colds, is also conflicting. Most of the reports state that the severity of colds is less in the group receiving vitamin A. Some of the reports state that the incidence of colds is also less.[129] Although the investigators attempted to secure adequate controls, it was usual to select volunteers for the experimental groups. The stated incidence of colds during a previous year was often compared with the observed incidence during the experiment. The subjective element was not always eliminated. In Cameron's study [130] this factor was recognized and her control subjects received a placebo of lactose. Cameron also calculated the daily intake of vitamin A. She found that, although the incidence of colds was not decreased by adding 5,000 international units of vitamin A to a diet already containing 4,300 units, the duration was decreased. The general criticisms just raised to this type of study have been met in the report of Shibley and Spies.[131] These authors studied groups of students selected by random sampling; no subject knew to which group he belonged; the record of infections was accurate. One group received 200,000 international units of vitamin A and 4,000 units of vitamin D per week; another received, as viosterol, 400 units of vitamin D, and the third received maize oil. The seasonal incidence of colds and their severity were alike in all groups. The duration of colds was significantly shorter in those students who received vitamin A.

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[122] Donaldson, S., and Tasker, J.: Preliminary Notes on the Treatment of Pneumonia with Vitamin A, Proc. Transvaal Mine Medical Officers Assn. **9:** 64, Nos. 107-108 (Feb.-March) 1930.

[123] Ellison, J. B.: Intensive Vitamin Therapy in Measles, Brit. M. J. **2:** 708 (Oct. 15) 1932.

[124] Orenstein, A. J.: Vitamin A in Treatment of Pneumonia, South African M. J. **6:** 685 (Nov. 12) 1932.

[125] Mackay, Helen M. M.; Linford, Hilda M.; Mitman, Maurice, and Wild, Mary H.: The Therapeutic Value of Vitamins A and D in Measles, Arch. Dis. Childhood **11:** 127 (June) 1936.

[126] Sutliff, W. D.; Place, E. H., and Segool, S. H.: Cod Liver Oil Concentrate (Concentrated Vitamins A and D): Ineffectiveness of Large Doses in the Prophylaxis of Otitis Media Complicating Scarlet Fever, J. A. M. A. **100:** 725 (March 11) 1933.

[127] Clausen, S. W.: The Exploitation of Vitamin A, New York State J. Med. **34:** 154 (Feb. 15) 1934.

[128] Sehestedt, H.: Die Beziehungen zwischen der Widerstandsfähigkeit der Kinder gegen Infekte der oberen Luftwege und dem Gehalt der Nahrung an Vitamin A, Monatschr. f. Kinderh. **63:** 351, 1935.

[129] Holmes, A. D.; Pigott, M. G.; Sawyer, W. A., and Comstock, L.: Vitamins Aid Reduction of Lost Time in Industry, J. Indust. & Engin. Chem. **24:** 1058 (Sept.) 1932; The Influence of Body Weight and the Administration of Cod Liver Oil on Industrial Absenteeism, J. Am. Dietet. A. **10:** 208 (Sept.) 1934; Cod Liver Oil: A Five Year Study of Its Value for Reducing Industrial Absenteeism Caused by Colds and Respiratory Diseases, Industrial Med. **5:** 359 (July) 1936. Beard, H. H.: The Prophylactic Effect of Vitamins A and D upon the Prevention of the Common Cold and Influenza, J. Am. Dietet. A. **10:** 193 (Sept.) 1934. Gardner, E. L., and Gardner, F. W.: Vitamin A and Colds, Am. J. [illegible] . J. [illegible] On the State of Health . . . of About 450 Brewery Workers and Officials, and of About 200 Soldiers: The Effect of a Daily Dose of Vitamins (Spinatin), Ztschr. f. Hyg. u. Infectionskr. **117:** 229 (July) 1935. Cameron.[130]

The evidence reviewed indicates that severe deficiency of vitamin A lowers resistance to infection in man and that the administration of vitamin A during the course of an infection probably has no beneficial effect on the outcome unless severe deficiency is present. There is evidence that moderate deficiency of vitamin A may also increase the duration or severity of respiratory infections in man; that an adequate intake of vitamin A may lessen the severity and duration of infection in persons moderately deficient. It is also evident that many other factors are of equal or greater influence on infection, and no justification exists for calling vitamin A "the anti-infective vitamin."

HYPERVITAMINOSIS: INJURIOUS EFFECTS ASCRIBED TO PREPARATIONS OF VITAMIN A

When large amounts of pure carotene in a suitable solvent are administered to animals by mouth or by injection, no harmful effects are observed,[132] although Lewis and Reti observed that the impure lipoid soluble extract of carrots is toxic. In human beings following the ingestion of large amounts of vegetables containing carotene, and also in persons suffering from certain metabolic disorders, especially diabetes, carotene may accumulate in the skin in amounts sufficient to cause a deep yellow color known as xanthosis cutis. The term "carotenemia," although frequently applied to this condition, is not an appropriate designation, for carotenoid pigments are usually present in the plasma of human blood, and in lipoid nephrosis a marked increase of the plasma carotene may exist[133] without xanthosis cutis. No harmful effects have been ascribed to hypercarotenemia, but xanthosis cutis may be mistaken for jaundice.

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[130] Cameron, Hazel C.: The Effect of Vitamin A upon Incidence and Severity of Colds Among Students, J. Am. Dietet. A. **11:** 189 (Sept.) 1935.

[131] Shibley, G. S., and Spies, T. D.: The Effect of Vitamin A on the Common Cold, J. A. M. A. **103:** 2021 (Dec. 29) 1934.

When large amounts of concentrated preparations of vitamin A are administered to certain animals, injurious effects are observed. It is likely that some of these effects are due to vitamin A, but satisfactory proof is lacking because pure preparations of vitamin A were not used. It has been shown that the injurious effects do not occur if the vitamin is destroyed in the preparations by oxidation or by ultraviolet irradiation. This argument is not convincing because these procedures may destroy some other toxic substance.

Drigalski[134] administered 0.5 and 1 cc. daily of a concentrate of fish liver oil ("Vogan"; 1 cc. = 40,000 rat units of vitamin A) to rats weighing 100 Gm. Their coats became rough; cachexia, catarrhal conjunctivitis, hemorrhagic rhinitis and diarrhea developed. Death occurred within five to eight days in the animals receiving the larger dose and within seven to nineteen days in those receiving the smaller dose. At autopsy Drigalski and Laubmann[135] found degeneration of the renal glomeruli and tubules, proliferation of the reticulo-endothelial cells of the spleen, absence of striations in some of the cardiac muscle fibers, degeneration of the testes, and only slight changes in the liver. Moll, Domagk and Laquer[136] report similar injuries in mice, and Fasold[137] calls attention to an elective fatty infiltration in the Kupffer cells of the liver. Collazo and Rodriguez[138] add to the symptoms exophthalmos and spontaneous fractures. The lesions of the bones suggested osteomalacia[139] but could not be prevented by large doses of vitamin D. Rossi[140] doubted the existence of "hypervitaminosis A"; but the dose of vitamin used by him was about 400 units a day for the rat, whereas the other investigators had used from 20,000 to 40,000 units a day.

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[132] Wells, H. G., and Hedenburg, O. F.: The Toxicity of Carotin, J. Biol. Chem. **27**: 213 (Oct.) 1916. Lewis, J. T., and Reti, L.: Sur l'hypervitaminose A et l'innocuité des fortes doses de provitamine A (Carotine cristallisé), Compt. rend. Soc. de biol. **118**: 577, 1935.

[133] Clausen, S. W., and McCoord, A. B.: Unpublished data.

[134] von Drigalski, W.: Ueber Schädigungen durch Vitamin A, Klin. Wchnschr. **12**: 308 (Feb. 25) 1933.

[135] von Drigalski, W., and Laubmann, W.: Ueber Schädigungen durch Vitamin A, Klin. Wchnschr. **12**: 1171 (July 29) 1933.

The literature which deals with injurious effects following the use of cod liver oil or of relatively small amounts of concentrates is very difficult to evaluate. Hopkins[141] found that an excess of cod liver oil in the diet of rats was injurious unless "balanced" by the addition of vitamin B complex. Following this observation, the theory of vitamin balance has developed,[142] but while accepting the observations as accurate, it is obvious that one must consider not only the relative amounts of vitamins but also the relative amounts of minerals and of other substances. As a rule the basic diets have been artificial ones, unusual for the species of animal being studied. Swedish workers report that cod liver oil, even in such small doses as 0.1 cc. per kilogram daily, may cause a variety of injurious effects, especially on the heart muscle, in mice, rats, rabbits, dogs, calves, cats, pigs, and human infants.[143] Norris and Church [144] have stated that the symptoms resemble those due to deficiency of vitamin B complex and may be due to isoamylamine in the small amount found in cod liver oil, but they failed to test their oil for the presence of this substance. Their view is not supported by the direct experiments of Bell, Gregory and Drummond,[145] who showed that a disturbance of growth in rats was produced by a diet containing 15 per cent of cod liver oil and could be made to disappear by an increase in the amount of vitamin B complex. The toxic substance in their cod liver oil could be extracted with hydrochloric acid but was not an amine. Turner, Meigs and Converse [146] stated that rabbits fed a grain mixture with low grade roughage have an even chance of surviving to an age of 180 days. If 0.7 Gm. of cod liver oil per kilogram is added, death will almost certainly occur between 90 and 150 days of age. If the roughage is of good quality alfalfa hay, little if any injury is produced by cod liver oil. On the other hand, calves receiving the grain mixture, poor quality roughage and skim milk do not survive unless cod liver oil (up to 0.7 Gm. per kilogram) is supplied but are injured by 2 Gm. per kilogram. Madsen and his co-workers [147] developed a synthetic diet containing cod liver oil but no hay or grain on which sheep and goats could be reared although the goats did not thrive. Rabbits and guinea pigs fed this diet did not survive. A constant symptom of failure to survive was paralysis due to degeneration of the skeletal muscles. The heart muscle was frequently involved. The substitution of a concentrate containing vitamins A and D for the cod liver oil delayed the onset and lessened the severity of the symptoms. The addition of cod liver oil (0.7 Gm. per kilogram daily) to a natural pasture diet caused death with the characteristic lesions in goats and sheep; but 0.1 Gm. of cod liver oil per kilogram daily caused no injury to animals on a normal diet. Cox and Roos [148] have repeated the experiments of Agduhr and Höjer. Although the rats in these experiments received doses of cod liver oil sixty times as large as the doses in Agduhr's experiments, Cox and Roos found no lesions in the heart. The whole question of degeneration of muscle and nervous tissue in animals consuming synthetic diets is at present the object of active study by several investigators. The questions of the presence of toxic substances and the absence of protective factors are involved. It appears at the present time that very little evidence exists that hypervitaminosis A is involved in the effect.

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[136] Moll, T.; Domagk, G., and Laquer, F.: Ueber das Vitamin A-Kozentrat "Vogan" und seine Wertbestimmung, Klin. Wchnschr. **12:** 465 (March 25) 1933.

[137] Fasold, H.: Zur Kenntnis der Lipoidanreicherungen nach grösseren Gaben an Vitamin A, Ztschr. f. d. ges. exper. Med. **94:** 35, 1934.

[138] Collazo, J. A., and Rodriguez, J. S.: Hypervitaminose A: Die Symptomatologie der durch Fütterung von reinem A-vitamin an jungen Ratten hervorgerufenen Hypervitaminose A, Klin. Wchnschr. **12:** 1732 (Nov. 4) 1933; Hypervitaminose A: II. Exophthalmus und Spontanfrakturen, ibid. **12:** 1768 (Nov. 11) 1933.

[139] Strauss, K.: Beobachtungen bei Hypervitaminose A, Beitr. z. path. Anat. u. z. allg. Path. **94:** 345 (Dec.) 1934.

[140] Rossi, G.: Gibt es eine Vitamin A-Hypervitaminose? Ztschr. f. Vitaminforsch. **2:** 194 (July) 1933.

[141] Hopkins, F. G.: The Present Position of the Vitamin Problem, Brit. M. J. **2:** 691 (Oct. 20) 1923.

[142] Harris, L. J., and Moore, T.: "Hypervitaminosis" and "Vitamin Balance," Biochem. J. **22:** 1461, 1928. Harris, L. J., and Moore, T.: Hypervitaminosis and Vitamin Balance: IV. An Instance of Vitamin Balance, ibid. **23:** 1114, 1929. Stepp, W., and Schröder, H.: Ueber den Antagonismus der Vitamine und seine Bedeutung für die Vitamintherapie, Klin. Wchnschr. **15:** 548 (April 18) 1936.

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[143] Agduhr, E.: Postnatal Development Under Different Conditions of Nutrition and Circumstances of Functioning: I. The Changes in the Heart Through the Presence of Cod Liver Oil (Oleum Jecoris Aselli) in the Food, Acta pædiat. **5:** 319, 1926; Changes in the Organism Caused by Cod Liver Oil Added to the Foods, ibid. **6:** 165, 1926. Höjer, A.: Changes in the Heart Through the Presence of Cod Liver Oil in the Food: Effect of Cod Liver Oil by Deficiency in Vitamin B, ibid. **6:** 180, 1926. Agduhr, E.: Are the So-Called A Vitamins in Cod Liver Oil the Cause of Its Toxic Effect on the Organism, and Can a Basal Diet Complete as Regards the So-Called B and C Vitamin Contents Prevent This Toxic Effect? ibid. **7:** 289, 1928; Zur Kenntnis der toxischen Nebenwirkung des Dorschlebertrans auf den Organismus. Monatschr. f. Kinderh. **47:** 97, 1930. Agduhr, E., and Stenström, N.: The Appearance of the Electrocardiogram in Heart Lesions Produced by Cod Liver Oil Treatment, Acta pædiat. **10:** 167, 1931.

[144] Norris, E. R., and Church, A. E.: The Toxic Effect of Fish Liver Oils and the Action of Vitamin B, J. Biol. Chem. **89:** 437 (Nov.) 1930.

[145] Bell, M. E.; Gregory, E., and Drummond, J. C.: Studies of the Alleged Toxic Action of Cod Liver Oil and Concentrates of Vitamin A, Ztschr. f. Vitaminforsch. **2:** 161 (July) 1933.

[146] Turner, W. A.; Meigs, E. B., and Converse, H. T.: Toxic Effect of Cod Liver Oil in the Ration of the Rabbit and the Calf, J. Biol. Chem. (Proc. Am. Soc. Biol. Chem.) **114:** civ (May) 1936.

[147] Madsen, L. L.; McCay, C. M., and Maynard, L. A.: Possible Relationship Between Cod Liver Oil and Muscular Degeneration of Herbivora Fed Synthetic Diets, Proc. Soc. Exper. Biol. & Med. **30:** 1434 (June) 1933. Madsen, L. L.; McCay, C. M.; Maynard, L. A.; Davis, G. K., and Woodward, J. C.: Synthetic Diets for Herbivora, with Special Reference to the Toxicity of Cod Liver Oil, Mem. 178, Cornell Univ. Agric. Exper. Sta., June 1935, p. 63.

Occasional untoward effects have been ascribed to cod liver oil given to patients. Czerny [149] gave 80 cc. daily to tuberculous children and frequently observed the development of seborrheic dermatitis of the face and scalp. Sensitivity to cod liver oil, resulting in urticaria or eczema, has been reported in four patients by Balyeat and Bowen [150] and to halibut liver oil in two cases by Pfister.[151]

In view of the general favorable clinical experience with the use of cod liver oil, one would have to be very sure that the evidence for its harmful effect on human beings is strong enough to be accepted. The literature quoted in this section is so contradictory as to afford, at the present time, no adequate evidence.

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148. Cox, W. M., Jr., and Roos, A. J.: On the Alleged Toxicity of Cod Liver Oil, Bull. Johns Hopkins Hosp. **54**: 430 (June) 1934.

149. Czerny, A.: Beitrag zur Lebertrantherapie, Therap. d. Gegenw. **14**: 49 (Feb.) 1912.

150. Balyeat, R. M., and Bowen, R.: Cod Liver Oil Sensitivity in Children, Am. J. Dis. Child. **47**: 529 (March) 1934.

151. Pfister, F. F.: A Skin Manifestation from the Excessive Administration of Vitamins, J. A. M. A. **102**: 533 (Feb. 17) 1934.

CHAPTER IV

VITAMIN A

METHODS OF ASSAY AND SOURCES IN FOOD

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The early recognition of the significance of vitamin A as an essential constituent of the diet of man made the quantitative as well as the qualitative identification of food sources an important problem for investigators engaged in research on nutrition. Although vitamin A was one of the first, if not the first, of the vitamins to be discovered, accurate quantitative methods of analysis have been slow of development. This can be explained by the fact that, although its chemical identity had been established with reasonable certainty, synthesis of the vitamin and its separation in crystalline form was accomplished only recently.[1] The situation has been further complicated by the necessity for adopting a device for expressing vitamin A potency, since obviously the values could not be expressed in terms of vitamin A. In some of the early studies attempts were made to find some product that might serve as a standard, but it soon became apparent that the vitamin A content of natural foods was so variable that the chances of finding a suitable substance were remote. Several "units" accordingly were suggested.

After the discovery had been made that carotene is converted into vitamin A by the animal organism, the Permanent Commission on Biological Standardization of the Health Organization of the League of Nations selected a well defined sample of carotene as the International Standard of Reference.[2] Although the selection of a substance of standard vitamin A potency has now been achieved, the preferred method of assay is still unsettled. In general the methods proposed are of three types: the so-called biologic methods, based on the behavior of laboratory animals; the color tests, and the more recently developed spectrographic, or absorption, methods. The biologic methods include the growth test; the ophthalmia test, based on the cure or prevention of the typical ocular condition due to a deficiency of vitamin A, and the vaginal smear test, which makes use of the irregular estrus and the appearance of cornified cells in the vaginal epithelium in vitamin A–deficient animals. The method of assay to be selected is dependent on the nature of the vitamin A activity, that is, on whether this activity is due to vitamin A itself, to provitamin A or to both, in the substance to be examined. The present discussion of methods will cover (1) the biologic methods, including the test based on the growth of rats and the vaginal smear test, (2) the color test with antimony trichloride and (3) the spectrographic method. The "growth" method is at present the official method of the U. S. Pharmacopeia[3] and it will be described first.

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[1] Wilke, Carl: Vitamin A Has Been Synthesized by Kuhn, Indust. & Engin. Chem., News. Ed. **29**: 291 (July 10) 1937. Holmes and Corbet.[46]

[2] League of Nations, Health Organisation: Second Conference on Vitamin Standardisation, London, June 12-14, 1934, Quart. Bull. Health Organ., League of Nations **3**: 428 (Sept.) 1934.

BIOLOGIC METHODS OF ASSAY

Early studies showed that the growth of experimental animals is proportional to the vitamin A content of the diet. It was also shown that vitamin A is concerned with the prevention and cure of a specific pathologic condition.

The Growth Method.—The assay of vitamin A by the growth method was introduced as early as 1920.[4] As developed and applied in various laboratories, this method embodies the following essential features: Young rats reared on a diet supplying vitamin A sufficient for growth and reproduction but not sufficient to permit excess storage are restricted to a diet which contains all the nutrients needed for growth except vitamin A. They are maintained on this diet long enough to reduce their body store of vitamin A until it will no longer support growth (preliminary, or depletion, period). The animals are then housed individually, and each is fed a specified daily allotment of the test material, as a supplement to the basal diet, throughout a period of several weeks (test period). During this time they are weighed at regular intervals, and there is a final weighing at the termination of the test. The increase in weight, or the growth response of the animal, obtained by subtracting from the final weight the weight on the day the first supplemental feeding was given, may then be used as a measure of the quantity of vitamin A ingested. Comparable, or matched, lots of animals given different quantities of vitamin A–containing material show growth graded to the dose. The relation between gain in weight and vitamin A intake is not linear but may be represented by a curve. This relationship has been rather extensively studied by Coward; she has derived formulas for the curve, which she calls the curve of response.[5]

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[3] United States Pharmacopeial Convention: The Pharmacopeia of the United States of America, Eleventh Decennial Revision, 1935.

[4] Drummond, J. C., and Coward, Katharine H.: Researches on the Fat-Soluble Accessory Factor (Vitamin A): VI. Effect of Heat and Oxygen on the Nutritive Value of Butter, Biochem. J. **14**: 734 (no. 6) 1920. Osborne, T. B., and Mendel, L. B.: Nutritive Factors in Plant Tissues: IV. Fat-Soluble Vitamine, J. Biol. Chem. **41**: 549 (April) 1920.

The growth method has been refined and standardized until it is possible to obtain results with it that have as high a degree of accuracy as can be expected of procedures in which animals are used as reagents. The details of the method as applied by different investigators lend themselves, however, to a variety of interpretations, since rules acceptable to the majority have not been established. Consideration must be given not only to selection of a basal diet devoid of vitamin A and adequate in all other factors but to such questions as age, weight and dietary history of the experimental animals; acceptable weight limits of animals at the end of the depletion period; criteria for determining when the animals are depleted and suitable for the test; length of test period required for reliable results; relative significance of results with males and with females; effect of feeding the supplements in multiples of the daily dose at periodic intervals rather than in single daily doses; number of test animals required in an assay group assigned a given quantity of test material in order that the average gain in weight may be accepted as reliable; number of groups of experimental animals receiving graded doses of supplement required to establish the relation between vitamin A intake and gain in weight. Because most of these variables affect the accuracy of the results obtained, it seems advisable to consider the results reported in regard to them.

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5. Coward, Katharine H.; Key, Kathleen M.; Dyer, F. J., and Morgan, Barbara G. E.: The Determination of Vitamin A, Biochem. J. **24**: 1952 (no. 6) 1930. Coward, Katharine H.: The Relation of the Growth Response to Dosage of Vitamin A: Confirmation of a Curve Relating Response to Dose of Vitamin A Given, ibid. **28**: 865 (no. 3) 1934. Coward, Dyer, Morton and Gaddum.[14]

The vitamin A–free diet in most common use consists of casein extracted with a suitable fat solvent or subjected to prolonged heating [6] to render it vitamin A-free, vitamin A–free starch, yeast to supply the water-soluble growth-promoting factors and a suitable salt mixture. Vitamin D may be supplied by irradiation of the oil, the starch or a portion of the yeast or through the daily administration of small quantities of irradiated cholesterol or ergosterol. Recently some investigators have added a vitamin A–free fat or oil carrying the growth-promoting unsaturated fatty acids.[7] Linseed oil or corn oil may be used for this purpose.

The colony from which the experimental animals are obtained should be maintained on a diet containing enough vitamin A to support growth and reproduction but not enough to permit excess storage by the young.[7a] Otherwise the depletion period will be unduly long and the animals will be too large when depleted. The time required for depletion may be kept at a minimum by giving mother and young the vitamin A–free food during the lactation period [8] or, if the mother's diet contains whole milk (powder), by substituting skimmed milk (powder) during some portion of the lactation period.[9] Animals suitable for test purposes are described on the basis of weight at the time of weaning,[10] or on the basis of weight at the end of the depletion period.[11] The best results seem to be obtained with animals weighing between 70 and 90 Gm. when depleted. Animals weighing more than 100 Gm. are generally considered unsuitable. Criteria for judging when the depletion period should be terminated cannot be given categorically but to a certain extent must be established by the investigator for the colony of animals with which he is working. The animals should show stationary or declining weight and may or may not have acquired ophthalmia. In any case, symptoms of extreme vitamin A deficiency should not be present.[12] The length of the test period used depends on the accuracy desired, the reliability increasing in general the longer the test is continued, at least up to eight weeks.[13] Male and female test animals cannot be used interchangeably at all levels of vitamin A intake. At the low levels they show similar rates of gain, but at higher levels the males grow faster.[14] Nine or ten animals in an assay group seem to be sufficient to give results that may be considered reliable,[15] although this number may vary somewhat according to the rate of gain[16] and the sex of the animals, twice as many females as males being required for the same degree of reliability.[17] The number of assay groups required to establish the relation between gain in weight and vitamin A intake depends on the reliability of the results from each group and the accuracy desired. Three points are naturally the least that can be used, and four or five are probably sufficient. The vitamin A supplements are generally specified in terms of the daily dose. The results seem to be the same, however, whether this is fed daily or in multiples of the daily dose at less frequent intervals.[18] A more recent report indicates that the biological method may be made to yield more reliable data through the use of statistical procedures.[18a]

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[6] Todhunter, E. N.: A Comparison of Heated Casein with Extracted Casein in the Basal Diet for the Determination of Vitamin A, J. Nutrition **13:** 469 (May) 1937.

[7] Evans, H. M., and Lepkovsky, Samuel: Vital Need of the Body for Certain Unsaturated Fatty Acids: III. Inability of the Rat Organism to Synthesize the Essential Unsaturated Fatty Acids, J. Biol. Chem. **99:** 231 (Dec.) 1932.

[7a] Davies, W. A., and Moore, T.: Vitamin A and Carotene, XV. The Influence of the Vitamin A Reserve on the Length of the Depletion Period in the Young Rat, Biochem. J. **31:** 172 (Jan.) 1937.

[8] Nelson, E. M.: Regulating the Storage of Vitamin A in Animals That Are to Be Used for the Determination of This Vitamin, Science **68:** 212 (Aug. 31) 1928.

[9] Unpublished data, United States Department of Agriculture, Bureau of Home Economics.

[10] Nelson.[8] U. S. Pharmacopeia.[3]

[11] Sherman, H. C., and Burtis, M. P.: Factors Affecting the Accuracy of the Quantitative Determination of Vitamin A, J. Biol. Chem. **78:** 671 (Aug.) 1928.

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[12] Norris and Church.[43] U. S. Pharmacopoeia.[3]

[13] Irwin, Margaret H.; Brandt, A. E., and Nelson, P. Mabel: Applications of Statistical Method to the Data of Vitamin Feeding Experiments: I. The per Cent Effect of Measured Variables, J. Biol. Chem. **88**: 449 (Sept.) 1930. Sherman and Burtis.[11] Coward.[16] Norris and Church.[43]

[14] Coward, Katharine H.; Dyer, F. J.; Morton, R. A., and Gaddum, J. H.: The Determination of Vitamin A in Cod Liver Oils (*a*) Biologically, (*b*) Chemically, (*c*) Physically, with a Statistical Examination of the Results, Biochem. J. **25**: 1102 (no. 4) 1931. Sherman and Burtis.[11]

[15] Sherman, H. C., and Batchelder, E. L.: Further Investigation of Quantitative Measurement of Vitamin A Values, J. Biol. Chem. **91**: 505 (May) 1931. Coward, Dyer, Morton and Gaddum.[14] Coward and Key.[18]

[16] Coward, Katharine H.: The Influence of the Length of the Test Period on the Accuracy Obtainable in a Vitamin A Test, Biochem. J. **27**: 445 (no. 2) 1933. Irwin, Brandt and Nelson.[13]

[17] Coward, Katharine H.: Variation in Growth Response of Rats in Vitamin A Tests Compared with the Variation in Rats Growing Normally, Biochem. J. **26**: 691 (no. 3) 1932.

[18] Coward, Katharine H., and Key, Kathleen M.: Simplification of Vitamin Tests: The Effect of Giving Doses Half-Weekly for Vitamin A Tests, and Once Only for Vitamin D Tests, Instead of Giving Doses Daily, Biochem. J. **28**: 870 (no. 3) 1934.

[18a] Swanson, P. P.; Stevenson, G. T., and Nelson, P. M.: A Method of Increasing Precision in Vitamin A Assay, J. Nutrition **15**: 103 (Feb.) 1938.

Interpretation of Results.—As already stated, the growth method was in use several years before the adoption of the International Standards of Reference for vitamins A, B_1, C and D. During that time several units were proposed for expressing vitamin A activity in terms of the measurements made. The one most extensively used was perhaps the Sherman, or the Sherman-Munsell, unit, sometimes called the rat-growth unit. This unit was defined as that amount (of vitamin A) which when fed daily (six times a week) just sufficed to support a rate of gain of 3 Gm. a week in a standard test animal (rat) during an experimental feeding period of from four to eight weeks.[19] To obtain values in terms of this unit, it was customary to use at least three groups of comparable ("matched") test animals: one to receive a daily supplement of test material designed to support the standard rate of gain of 3 Gm. a week, one to receive a quantity of supplement somewhat less than this and the third to receive a quantity somewhat larger. At the end of the test the average gain in weight for each group was calculated, and a curve was drawn relating quantity of test food ingested to gain in weight. The quantity of test material required to support a gain in weight of 3 Gm. a week was then derived from this curve. The reciprocal of this value (expressed in grams) indicated the number of "rat growth" units of vitamin A per gram of material.

Although the rat growth unit was soon recognized as of little use for expressing absolute vitamin A values, the 3 Gm. a week rate of gain is often used as representative of the most reliable and most sensitive portion of the curve relating gain in weight of rats to vitamin intake.

Since adoption of the International Standards of Reference[20] for the vitamins it has been advisable to carry out biologic assays in such a way that the quantitative estimates derived from the results may be expressed in terms of international units rather than in terms of "rat growth" units.[21] Failure to give serious attention to the necessity of considering devices for making accurate comparisons with the international standards has in many instances led to the derivation of estimates that were far from the true values. Two methods of carrying out assays to obtain exact values in terms of international units have been suggested and described in detail.[22]

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[19] Sherman, H. C., and Munsell, Hazel E.: The Quantitative Determination of Vitamin A, J. Am. Chem. Soc. **47**: 1639 (June) 1925.

[20] A detailed discussion of the selection of the international standard carotene and the use of a selected sample of cod liver oil as a subsidiary standard is given in a Special Report of the Medical Research Council. Information on the selection of a suitable solvent for the standard is included. This material, presented by Hume and Chick,[57] has been summarized by E. V. McCollum (The Fat-Soluble Vitamins, in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press, 1936, p. 379).

Attempts have been made to derive a factor for converting vitamin A values expressed in Sherman or rat growth units to equivalent values expressed in international units.[22] In this connection it is essential to emphasize the fact that the rat growth unit has no absolute value in terms of vitamin A and that, therefore, the derivation of a conversion factor is not a valid procedure. Any implied relationship must be considered as merely indicative. Values have appeared in the published literature from time to time, to be sure, but these have included extremes of 0.5 and 1.5 and there has been no basis for selecting one in preference to another. As a matter of fact, for some data reported one factor would probably more nearly serve the purpose, while in other cases the value at the other extreme might better be used.

The illogic of attempting to derive a single conversion factor of this sort may be illustrated by a consideration of factors suggested by results obtained during the past year[23] with positive control animals used in vitamin A assays on various products. All of these animals were given daily during a five week test period an amount of U. S. P. reference oil estimated to supply 1 international unit of vitamin A on the basis of the vitamin A value claimed for this oil. A total of sixty-five litters of animals were represented and all were drawn from the colony especially maintained for vitamin A assay work. Since in assays of this kind results with six to ten animals are generally considered sufficient to give an average that may be accepted as reliable, average gains in weight were derived for groups made up respectively of the controls taken from eight litters in order of the date of birth of the animals. The resulting average gains in weight for the eight groups established in this way were 18.0, 33.5, 30.3, 23.5, 16.4, 17.3, 31.3 and 28.3 Gm. By reference to a dose-effect curve for cod liver oil these were estimated to represent approximately the following vitamin A values in Sherman (Munsell) units: 0.9, 2.5, 2.1, 1.4, 0.8, 0.9, 2.2 and 1.9. On the basis of these results, therefore, the number of Sherman (Munsell) units equivalent to 1 international unit might be anything between 0.8 and 2.5. The use of another dose-effect curve might give slightly different values for Sherman units, of course, but the average gains in weight still stand as evidence of the variability in the value of the "growth unit" even in tests carried out with presumably "standard" animals.

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[21] The Permanent Commission on Biological Standardisation of the Health Organisation of the League of Nations at its 1931 conference (Report of the Conference on Vitamin Standards, London, June 17-20, 1931, Geneva, 1931, p. 68) adopted a specially prepared sample of carotene as the International Standard of Reference for vitamin A. A unit of vitamin A (international unit) was then defined as the vitamin A activity of 1 microgram of this international standard. During the interval from 1931 to 1934, when the next conference was held,[2] it was learned that the various forms of carotene do not possess the same vitamin A activity, β-carotene being more active than either α-carotene or γ-carotene. Accordingly, at the 1934 international conference a sample of pure β-carotene was adopted to replace the 1931 standard, which had been shown to be a mixture. The sample of β-carotene had been carefully assayed against the old standard, and from the average results from several laboratories, 0.6 microgram was accepted as equivalent to 1 microgram of the 1931 standard. The international unit of vitamin A is therefore the vitamin A activity of 0.6 microgram of pure β-carotene.

[22] Daniel, Esther P., and Munsell, Hazel E.: Vitamin Content of Foods: A Summary of the Chemistry of Vitamins, Units of Measurement, Quantitative Aspects in Human Nutrition, and Occurrence in Foods, Misc. Pub. 275, United States Department of Agriculture, Bureau of Home Economics, 1937.

The Single-Feeding Growth Method.—The growth method as usually carried out involves the feeding of supplements at periodic intervals during several weeks and the estimation of quantitative values from the gain in weight of the animals during the test period. A somewhat modified form of this technic is the so-called single feeding method, in which a single dose of vitamin A–bearing material is given to vitamin A–depleted animals. In this case the survival of the animals is proportional to the vitamin A content of the material fed.[24] By measuring the area under the curve relating weight to survival days of animals fed different materials, it is possible to compare the vitamin A potency of these materials.[25] This method was recently successfully applied in the assay of pasture grasses.[26]

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[23] Unpublished data, Bureau of Home Economics, United States Department of Agriculture.

[24] Sherman, H. C., and Cammack, M. L.: A Quantitative Study of the Storage of Vitamin A, J. Biol. Chem. **68**: 69 (April) 1926. Nelson, E. M.; Walker, Reed, and Jones, D. B.: Determination of Vitamin A by a Preventative Method, J. Biol. Chem. **92**: vi (June) 1931.

The Vaginal Smear Method.—Female rats deprived of vitamin A show abnormal estrus and the presence of cornified cells in the vaginal epithelium.[27] This condition, which is one of the first and most pronounced evidences of a lack of vitamin A, clears up directly on the administration of vitamin A. Studies have been made to determine whether this phenomenon could be made the basis of a quantitative method.[28] Although the method developed has several distinct advantages, it does not commend itself for general adoption, because of the rather tedious procedure and the difficulty involved in interpreting the results quantitatively.[29]

CHEMICAL AND PHYSICAL METHODS

As information was accumulated on the chemical and the physical characteristics of vitamin A, certain properties were identified that offered possibilities as bases for methods of quantitative analysis. The more extensively studied methods include the color tests, particularly the blue color reaction with antimony trichloride, and methods based on the selective absorption shown by vitamin A and vitamin A compounds. Although the reaction with antimony trichloride is no longer considered sufficiently specific to serve as the basis of a dependable quantitative method, it may be used for certain routine purposes of control and therefore will be reviewed briefly. The spectrographic method of analysis holds promise of eventually becoming an accepted technic for quantitative work, although it is still largely in the investigative stage.

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[25] Sherman, H. C., and Todhunter, E. N.: The Determination of Vitamin A Values by a Method of Single Feedings, J. Nutrition **8:** 347 (Sept.) 1934.

[26] Hodgson, R. E., and Knott, J. C.: The Method of Single Feeding as Used in Measuring the Vitamin A Activity of Pasture Grass Artificially Dehydrated at Different Temperatures, Am. Dairy Sc. A. West. Div. 22d Annual Meeting Proc., 1936, p. 41.

[27] Evans, H. M., and Bishop, K. S.: On the Existence of a Hitherto Unrecognized Dietary Factor Essential for Reproduction, Science **56:** 650 (Dec. 8) 1922.

[28] Macy, Icie G.; Outhouse, Julia; Long, M. Louisa, and Graham, Alice: Human Milk Studies: I. Technique Employed in Vitamin Studies, J. Biol. Chem. **73:** 153 (May) 1927. Coward, Katharine H.: The Influence of Vitamin A Deficiency on the Oestrous Cycle of the Rat, J. Physiol. **67:** 26 (Feb. 28) 1929. Coward, Katharine H.; Morgan, Barbara G. E., and Dyer, F. J.: II., ibid. **69:** 349 (May) 1930.

[29] Baumann, C. A., and Steenbock, Harry: The Vaginal Smear Method of Determining Vitamin A, Science **76:** 417 (Nov. 4) 1932.

The color test and the spectrographic method as reviewed here do not apply to the determination of vitamin A activity due to the carotenoid precursors. No attempt will be made in the limited space allotted to this paper to discuss the blue reaction with antimony trichloride or the spectrographic technic as applied to the determination of the carotenoid precursors. The international unit, on the other hand, is defined in terms of the international stand, which is β-carotene. Thus it is not possible to interpret results from the color test or the spectrographic technic directly in terms of international units by comparison with results of concurrent tests on the international standard. It is necessary to use conversion factors giving the relation between the potency of vitamin A expressed in international units as determined by the biologic method and the constant for vitamin A of the chemical or physical property being measured. In several reports in which conversion factors are discussed, values determined by the biologic method and expressed in terms of international units are referred to as "biologic values." This expression should not be confused with the Sherman, or rat growth, unit of vitamin A, which is sometimes called the biologic unit.

The Color Test with Antimony Trichloride.—As early as 1920[30] it was suggested that the color reaction of cod liver oil with concentrated sulfuric acid might be related to the vitamin A content. Systematic investigations soon identified several color reagents, including arsenic trichloride,[31] which gave a brilliant ultramarine color with cod liver oil. The evidence obtained seemed to support the conclusion that this reaction was specific for vitamin A. A number of the color reagents were investigated by Carr and Price,[32] who gave particular attention to conditions necessary for the determination of quantitative relationships. Of the reagents studied, antimony trichloride in chloroform solution was the most satisfactory. The authors gave detailed directions for carrying out the test, and for this reason it is often referred to as the Carr-Price color test. As a quantitative method it has been used chiefly with liver oils, concentrates and extracts. In applying the test to the assay of oils,[33] a 20 per cent solution of the oil in chloroform is prepared at a given temperature, usually 20 C. Of this solution 0.2 cc. is placed in a glass cell having an internal measurement of 1 cm., and the cell is placed in a colorimeter or tintometer designed for matching the color against standard glasses. Two cc. of antimony trichloride reagent (antimony trichloride in dry chloroform saturated at 20 C.) is added so that the two solutions mix. The blue color develops rapidly and then fades. Readings are taken as the color develops, the point of maximum intensity being taken as the correct value. The values read are usually expressed as blue units, or Lovibond blue units.

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[30] Rosenheim, Otto, and Drummond, J. C.: On the Relation of the Lipochrome Pigments to the Fat-Soluble Accessory Food Factor, Lancet **1**: 862 (April 17) 1920.

[31] Rosenheim, Otto, and Drummond, J. C.: A Delicate Colour Reaction for the Presence of Vitamin A, Biochem. J. **19**: 753 (no. 4) 1925.

[32] Carr, F. H., and Price, E. A.: Colour Reactions Attributed to Vitamin A, Biochem. J. **20**: 497 (no. 3) 1926.

Intensive studies of the antimony trichloride reaction with oils have shown that the color produced is not proportional to the amount of oil used, i. e., to the concentration of vitamin A.[34] The color reaction is not a linear function of vitamin A concentrations of oil. Furthermore, there seems to be no uniformity in the type of curve relating concentration of oil to color produced.[35] Results obtained with the unsaponifiable fraction, on the other hand, give a more nearly linear relationship.[36] These values also check satisfactorily with values obtained by the biologic method.[37]

Certain plant pigments, including carotene, are known to give a blue color reaction with antimony trichloride. The reaction with carotene may be differentiated from that with vitamin A by heating the solutions on a water bath at 60 C. The blue color with carotene will persist, while that with vitamin A changes to rose, violet-red or wine-red, according to the concentration of vitamin present.[38]

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[33] Antimony Trichloride Test for Cod Liver Oil, British Pharmacopeia, 1932, p. 596.

[34] (a) Wokes, F., and Willimott, S. G.: A Study of Antimony Trichloride as a Possible Quantitative Reagent for Vitamin A, Analyst **52**: 515, 1927. (b) Norris, E. R., and Danielson, I. S.: Comparison of Biological and Colorimetric Assays for Vitamin A as Applied to Fish Oils, J. Biol. Chem. **83**: 469 (Aug.) 1929. Norris and Church.[35]

[35] Norris, E. R., and Church, A. E.: A Study of the Antimony Trichloride Color Reaction for Vitamin A: II. The Dilution Curve of Cod Liver Oil with Antimony Trichloride Reagent, J. Biol. Chem. **87**: 139 (May) 1930.

[36] Norris, E. R., and Church, A. E.: A Study of the Antimony Trichloride Color Reaction for Vitamin A: I. J. Biol. Chem. **85**: 477 (Jan.) 1930. Smith, E. L., and Hazley, Violet: The Reaction of Antimony Trichloride with Cod Liver Oil and Its Unsaponifiable Fraction, Biochem. J. **24**: 1942 (no. 6) 1930. Coward, Dyer, Morton and Gaddum.[14] Wise and Heyl.[37].

[37] Wise, E. C., and Heyl, F. W.: Vitamin A Colorimetric and Biological Assay, J. Am. Pharm. A. **21**: 1142 (Nov.) 1932. Andersen, A., and Nightingale, E.: Test for Vitamin A in Margarine, Butter, and Other Fatty Foods, J. Soc. Chem. Ind. **48**: 139T (June 14) 1929. Coward, Dyer, Morton and Gaddum.[14] Norris and Church.[36] Smith and Hazley.[36] Lathbury.[45]

The blue reaction with vitamin A is more stable at low temperatures[39] and may be stabilized by the addition of hydroquinone[40] or guaiacol.[41] Certain other substances interfere with the reaction. A large number of these have been identified and tabulated according to the maximum quantity that may be present without masking the test.[42]

Interpretation of Results.—The relation of the Lovibond blue unit to the Sherman unit of vitamin A has been variously given. The wide discrepancies in the following factors indicate the futility of attempting to select a conversion factor:

1 Sherman unit = 2.18 Lovibond blue units[34b]

10 Sherman units (eight week test) = 7.1 and 8.2 Lovibond blue units.[43]

An attempt to establish a factor for interpreting Carr-Price values in terms of international units fares little better. The rich concentrate of vitamin A studied by Carr and Jewell[44] gave a blue value of 78,000 and contained approximately 1.6×10^6 international units of vitamin A per gram, giving a ratio between international units per gram and the blue value of approximately 21 to 1. Other work[45] indicates a ratio of 20 to 1 for the vitamin A of a distilled oil, and for natural oil the ratio is about 40 to 1. The crystalline vitamin A recently described by Holmes and Corbet[46] gave a blue value of 100,000 and has since been reported to contain approximately 3×10^6 international units per gram, indicating a ratio of 30 to 1. A more recent report further emphasizes the variability in the relation between these two measurements.[46a]

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[38] Andersen, A. C., and Levine, V. E.: Reaction to Differentiate Vitamin A from Carotene by Means of Antimony Trichloride, Proc. Soc. Exper. Biol. & Med. **32**: 737 (Feb.) 1935.

[39] Ender, Fredrik: The Reaction of Fish-Liver Oils with Antimony Trichloride, Biochem. J. **26**: 1118 (no. 4) 1932.

[40] Huston, R. C.; Lightbody, H. D., and Ball, C. D., Jr.: Some Biochemical Relations of Phenols: II. The Effect of Hydroquinone on the Vitamin A Content of Stored Oils, J. Biol. Chem. **79**: 507 (Oct.) 1928.

[41] Rosenthal, Eugene, and Erdelyi, John: Some Remarks Concerning the New Colour Reaction of Vitamin A, Biochem. J. **29**: 2112 (Sept.) 1935.

[42] Corbet, Ruth E.; Geisinger, Helen H., and Holmes, H. N.: Substances Which Interfere with the Antimony Trichloride Test for Vitamin A, J. Biol. Chem. **100**: 657 (May) 1933.

[43] Norris, E. R., and Church, A. E.: A Study of the Antimony Trichloride Color Reaction for Vitamin A: V. Evaluation of a Colorimetric Unit on the Basis of the Biological Unit for Vitamin A, J. Nutrition **5**: 495 (Sept.) 1932.

[44] Carr, F. H., and Jewell, William: Characteristics of Highly Active Vitamin A, Nature **131**: 92 (Jan. 21) 1933.

[45] Lathbury, Kathleen C.: Vitamin A Determination: Relation Between the Biological, Chemical and Physical Methods of Test, Biochem. J. **28**: 2254 (no. 6) 1934.

Spectrographic, or Absorption, Methods.—Use of the spectroscope has recently come to the fore as an important method of analysis, especially for the identification of substances occurring in such small quantities as to defy the ordinary methods of quantitative chemical analysis. If a substance shows selective absorption with characteristic maximums, it is a relatively simple matter to detect its presence and by determining the degree of absorption to obtain an estimate of the quantity present. This method has been applied in the case of vitamin A and vitamin A compounds with considerable success and promises to become an accepted method of analysis. For the most part the conventional types of equipment suitable for determining and measuring absorption in the visible and in the ultraviolet have been used, and the results have accordingly been referred to as spectroscopic, spectrographic or spectrophotometric measurements, according to the instrument employed.

Absorption of the Blue Antimony Trichloride–Vitamin A Compound.—The compound formed by antimony trichloride with the vitamin A of liver oils shows two absorption bands, with maximums near 606 and 572 millimicrons, respectively.[47] In the case of concentrates there may be considerable shift toward the red end of the spectrum, the maximums appearing near 617 and 583 millimicrons, although on dilution to give a vitamin A potency equivalent to that of cod liver oil

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46. Holmes, N. H., and Corbet, Ruth E.: A Crystalline Vitamin A Concentrate, Science **85**: 103 (Jan. 22) 1937.

46a. Robinson, F. A.: Studies on the Stability of Vitamins A and D. I, Biochem. J. **32**: 807 (May) 1938.

47. Drummond, J. C., and Morton, R. A.: Observations on the Assay of Vitamin A, Biochem. J. **23**: 785 (no. 4) 1929. Coward, Katharine H.; Dyer, F. J., and Morton, R. A.: The Determination of Vitamin A in Cod Liver Oils, (*a*) Biologically, (*b*) Chemically, (*c*) Physically, with a Statistical Examination of the Results: II. Further Evidence That the Intensity of Absorption at 328 Millimicrons Gives the Best Agreement with the Biological Measure of Vitamin A in Cod Liver Oils, ibid. **26**: 1593 (no. 5) 1932. Morton, Heilbron and Thompson.[48] Coward, Dyer, Morton and Gaddum.[14]

the shift is not apparent.[48] It has been suggested that liver oils may contain two chromogens giving a blue color with antimony trichloride, one showing the maximum at 606 millimicrons and the other the maximum at 572 millimicrons.[49] Under suitable conditions both of these bands are present. It is possible, however, by changing the concentration or the temperature or by adding certain chemicals to alter these conditions so that either one or the other of these bands, but not both, is shown.[50] There is still some difference of opinion as to the specificity of these bands for vitamin A.[51]

The difference in behavior of the 617 millimicron and the 583 millimicron bands of the concentrates may be construed to indicate lack of homogeneity in vitamin A concentrates unless a satisfactory explanation of the difference is found.[52]

The antimony trichloride blue reaction with vitamin A has repeatedly been observed to show a maximum near 693 millimicrons and other bands have been seen occasionally at 635-640 millimicrons. Certain work suggests that another vitamin akin to vitamin A and referred to as vitamin A_2 or factor A_2 may be responsible for the 693 millimicrons band.[52a] Evidence indicates that factor A_2 is a higher homologue of vitamin A_1 and that it has biological activity similar to that attributed to vitamin A_1.[52b]

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48. Morton, R. A.; Heilbron, I. M., and Thompson, Albert: Spectrographic Data Concerning Vitamin A and Liver Oils, Biochem. J. **25:** 20 (no. 1) 1931. Heilbron, I. M.; Heslop, R. N.; Morton, R. A.; Webster, E. T.; Rea, J. L., and Drummond, J. C.: Characteristics of Highly Active Vitamin A Preparations, ibid. **26:** 1178 (no. 4) 1932.

49. Gillam, A. E., and Morton, R. A.. The Antimony Trichloride Colour Test and the Ultraviolet Absorption of Liver Oils and Concentrates, Biochem. J. **25:** 1346 (no. 4) 1931. Heilbron, I. M.; Gillam, A. E., and Morton, R. A.: Specificity in Tests for Vitamin A: A New Conception of the Chromogenic Constituents of Fresh and Aged Liver Oils, ibid. **25:** 1352 (no. 4) 1931. Brode and Magill.[50] Morton.[52]

50. Brode, W. R., and Magill, Mary A.: A Critical Study of the Antimony Trichloride Color Test for Vitamin A, J. Biol. Chem. **92:** 87 (June) 1931. Morton.[52] Notewarp, O., and Weedon, H. W.: Spectrographic Studies on the Antimony Trichloride Reaction for Vitamin A. II. Influence of Oxidizing Reagents on the Reaction, Biochem. J. **32:** 1054 (June) 1938.

51. Magill, Mary A., and Brode, W. R.: A Comparison Between the Spectrophotometric and Biological Assay for the Vitamin A Content of Fish Liver Oils, Ohio J. Science **33:** 127 (March) 1933. Coward, Dyer, Morton and Gaddum.[14] Drummond and Morton.[47] Gillam and Morton.[49] Brode and Magill.[50] Morton.[52] Morgan, Edisbury and Morton.[56]

52. Morton, R. A.: The use of β-Methylindole in the Antimony Trichloride Colour Test for Vitamin A, Biochem. J. **26:** 1197 (no. 4) 1932.

52a. Edisbury, J. R.; Morton, R. A.; Simpkins, G. W., and Lovern, J. A.: The Distribution of Vitamin A and Factor A_2.I, Biochem. J. **32:** 118 (Jan.) 1938. Edisbury, J. R.; Morton, R. A., and Simpkins, G. W.: A Possible Vitamin A_2, Nature **140:** 234 (Aug. 7) 1937.

52b. Gillam, A. E.: The Vitamin A_1 and A_2 Contents of Mammalian and Other Animal Livers, Biochem. J. **32:** 1496 (Sept.) 1938.

Absorption of Vitamin A.—In 1925 Takahashi[53] described a highly active vitamin A preparation (biosterin) showing selective absorption in the ultraviolet with a maximum in the region of 320 millimicrons. Subsequent studies on oils and concentrates showed a high correlation between the vitamin A content as determined by the biologic method and absorption in the region having a maximum at 328 millimicrons.[54] In the case of oils, however, not all of the absorption at 328 millimicrons is due to vitamin A. The extraneous absorption varies from oil to oil and in one case was shown to be as high as 40 per cent.[55] Reliable data therefore are obtained only by making the test with the unsaponifiable fraction. Although the measurement of absorption in the ultraviolet gives a good indication of vitamin A activity, there are still certain discrepancies that must be explained before the method can be generally adopted.[56]

Application of the Spectrographic Method to the Quantitative Estimation of Vitamin A.—As implied in the foregoing discussion, the spectrographic method for vitamin A involves measurement of absorption in the region of the spectrum where vitamin A or vitamin A compounds show selective absorption. The measurement is usually made at or near the maximum of the particular absorption band used. This value expressed as a fraction of the value for 100 per cent vitamin A then indicates the proportion of vitamin A present. The validity of this relationship depends on whether Beer's law holds for all concentrations of vitamin A; i. e., whether the ratio of the coefficient of absorption to vitamin A concentration is a constant for all concentrations of vitamin A. In the application of the test this has been assumed to be the case.

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[53] Takahashi, K.; Nakamiya, Z.; Kawakami, K., and Kitasato, T.: On the Physical and Chemical Properties of Biosterin (a Name Given to Fat-Soluble A) and on Its Physiological Significance, Inst. Phys. Chem. Res. (Tokyo) Sc. Papers **3:** 81 (no. 32) 1925.

[54] Morton, R. A., and Heilbron, I. M.: The Absorption Spectrum of Vitamin A, Biochem. J. **22:** 987 (no. 4) 1928. Macwalter, R. J.: Some Factors Which Affect the Assay of Vitamin A by the Spectrographic Method, Biochem. J. **28:** 472 (no. 2) 1934. Coward, Dyer, Morton and Gaddum.[14] Drummond and Morton.[47]

[55] Crews, S. K., and Cox, S. J.: The Relationship between the Carr-Price Value and the 328 Millimicron Absorption Coefficient of Preparations Containing Vitamin A, Analyst **59:** 85 (Feb.) 1934. Coward, Dyer, Morton and Gaddum.[14]

[56] Morgan, R. S.; Edisbury, J. R., and Morton, R. S.: A Discrepancy Between Biological Assays and Other Methods of Determining Vitamin A: I. Biochem. J. **29:** 1645 (July) 1935. Pritchard, Harry; Wilkinson, Harry; Edisbury, J. R., and Morton, R. A.: II. ibid. **31:** 258 (Feb.) 1937. Wilkie.[60]

It has become customary in work with vitamin A to determine the coefficient of absorption on the basis of a 1 per cent solution of 1 cm. depth. The values are usually expressed as $E_{1\,\text{cm.}}^{1\%}$, where E represents the optical constant, $\log \frac{I_0 \text{ (intensity of incident light)}}{I \text{ (intensity of emergent light)}}$. The wavelength at which the determination is made must be indicated. This value is sometimes referred to as the specific absorptive index or the extinction coefficient and has been shown to vary somewhat depending upon the solvent used in making up the solution of vitamin A from which the reading is made.[56a]

When the extinction coefficient has been determined either for direct absorption in the ultraviolet in the region from 320 to 330 millimicrons or for the antimony trichloride blue compound at either of the two maximums specific for this compound, the next problem is to convert the reading into vitamin A potency in terms of international units. To do this it is necessary to know the value for international units per gram divided by the index coefficient for 100 per cent vitamin A, which is the factor by which the coefficient of absorption must be multiplied to obtain the vitamin A potency in international units. Reference has already been made to the rich concentrate of vitamin A described by Carr and Jewell.[44] This substance was shown to have coefficients of absorption for the blue reaction of $E_{1\,\text{cm.}}^{1\%}$ 617 millimicrons = 5,000 and $E_{1\,\text{cm.}}^{1\%}$ 580 millimicrons = 2,600 and a coefficient of absorption for absorption in the ultraviolet of $E_{1\,\text{cm.}}^{1\%}$ 328 millimicrons = 1,600. Six ten-thousandths of a milligram contained slightly more than 1 international unit; i. e., the substance had a biologic value between 1.6 and 2 million units per gram. On the basis of these constants the ratio

$$\frac{\text{biologic value (international units per gram}}{E_{1\,\text{cm}}^{1\%}\ 328} \qquad \frac{1.6 \text{ to } 2 \text{ million international units per gram}}{1{,}600}$$

would give a factor of approximately 1,250.

[56a] Gillam, A. E., and Ridi, M. S. E.: The Variation of the Extinction Coefficient of Vitamin A with Solvent, Biochem. J. **32**: 820 (May) 1928.

Other factors have been similarly determined on the basis of measurements made on rich concentrates of vitamin A. Those proposed up to 1935 are reviewed by Chick and Hume,[57] who give the accepted value for the conversion factor as 1,600. A more recent report by Hume [58] indicates that this factor is still the most satisfactory one to use.

From results of assays of fish liver oils by the biologic and spectrographic methods, Emmett and Bird [59] stated the conclusion that the ratio of

$$\frac{\text{international units per gram}}{E_{1\ \text{cm.}}^{1\%}\ 328\ \text{millimicrons}}$$

for vitamin A is "close to 2,000." In the case of concentrates the conversion factor varied with the quantity of alkali used in saponification.

Reference has already been made to the report of Holmes and Corbet [46] on the separation of crystalline vitamin A. This substance gave a value for $E_{1\%}^{1\ \text{cm.}}$ 328 millimicrons of 2,000. If the biologic value proves to be 3,000,000 international units, as tentatively reported, the conversion factor would be nearer 1,500 than 1,600.

The validity of the choice of the factor 1,600 or, for that matter, of any fixed factor for converting spectrographic readings to international units has been discussed by Wilkie.[60]

The determination by British investigators of the vitamin A in butter may be cited as an example of the application of the spectrographic method to the quantitative estimation of vitamin A.[61] The spectrophotometric method as applied to fish liver oils gives results comparable to those obtained by the chemical or biological methods.[61a]

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[57] Hume, E. Margaret, and Chick, Harriette: Reports on Biological Standards: IV. The Standardisation and Estimation of Vitamin A, Medical Research Council, Special Report Series, no. 202, London, His Majesty's Stat. Off., 1935.

[58] Hume, E. M.: Estimation of Vitamin A, Nature **139**: 467 (March 13) 1937.

[59] Emmett, A. D., and Bird, O. D.: Comparative Biological Value of Vitamin A as an Alcohol and Ester, J. Biol. Chem. **119**: xxxi (June) 1937.

[60] Wilkie, J. B.: Report on Vitamin A: Determinations with the Hilger Vitameter, J. A. Off. Agric. Chem. **20**: 208 (May) 1937.

[61] Morton, R. A., and Heilbron, I. M.: Vitamin A of Butter, Biochem. J. **24**: 870 (no. 4) 1930. Gillam, A. E.; Heilbron, I. M.; Morton, R. A.; Bishop, Gerald, and Drummond, J. C.: Variations in the Quality of Butter, Particularly in Relation to the Vitamin A, Carotene, and Xanthophyll Content as Influenced by Feeding Artificially Dried Grass to Stall-Fed Cattle, ibid. **27**: 878 (no. 3) 1933. Gillam, A. E.; Heilbron, I. M.; Ferguson, W. S., and Watson, S. J.: Variations in the Carotene and Vitamin A Values of the Milk Fat (Butter) of Cattle of Typical English Breeds, ibid. **30**: 1728 (Sept.) 1936.

Special Instruments.—The type of equipment generally used in spectrophotometric work is expensive and requires, furthermore, a skilled operator to obtain accurate results. Early in 1933 Adam Hilger, Ltd., London, placed on the market a simplified form of the spectrophotometer, called the vitameter. This instrument, which has been described by Irish,[62] is made especially for the assay of vitamin A and is calibrated to give $E_{1\,\text{cm.}}^{1\%}$ 328 millimicron values directly. A study of results obtained with the vitameter has been made by Wilkie,[60] who recommended that persons using the instrument interpret the readings by means of a dilution curve determined for the reference cod liver oil.

A modified spectrophotometer for the assay of vitamin A was recently described by McFarlan, Reddie and Merrill.[63] This instrument is small and compact, is claimed to be easy to operate and to give results in a minimum of time, about one and one-half minutes being required for a reading. The readings are given directly for log $\frac{I_0}{I}$ at 330 millimicrons, the wavelength of the monochromatic light used. A value of 1.32 for $E_{1\,\text{cm.}}^{1\%}$ 330 millimicrons for the U. S. P. reference cod liver oil was obtained. On the basis of 3,000 international units per gram given for this oil, the conversion factor for changing readings to international units was derived as 2,270.

FOOD SOURCES OF VITAMIN A

The study of food sources of vitamin A claimed the attention of investigators from the very time of discovery of this vitamin. As a result, considerably more data have accumulated on the occurrence of this factor in foods than is the case with some of the other vitamins. This material has been brought together in a publication recently issued by the United States Department of Agriculture[22] and will not be reviewed in detail here.

[61a] Holmes, A. D.; Black, A.; Eckler, C. R.; Emmett, A. D.; Heyl, F. W.; Neilsen, C., and Quinn, E. J.: Report of the Vitamin Assay Committee of the American Drug Manufacturers' Association—Twenty-Sixth Annual Meeting—May 1937. I. The Practical Application of the Spectrophotometric Method for Assay of Vitamin A, J. Am. Pharm. Assoc. **26:** 525 (June) 1937.

[62] Irish, F. W.: Report on Vitamin A: Determinations with the Hilger Vitameter, J. A. Off. Agric. Chem. **19:** 244 (May) 1936.

[63] McFarlan, R. L.; Reddie, J. W., and Merrill, E. C.: A New Photoelectric Method for Measuring Vitamin A, Indust. & Engin. Chem. **29:** 324 (July 15) 1937.

Foods are often classified from the standpoint of vitamin potency as fair, good or excellent sources, without any clear indication as to the actual meaning of these terms. In order to have uniform terminology in the Bureau of Home Economics for expressing relative significance of foods as sources of vitamins, these qualitative terms have been tentatively defined by limiting values expressed in Sherman units per hundred grams. The bases of the definitions are given here so that there may be no uncertainty as to the meaning of the text that follows. A food source containing 19 or less Sherman units of vitamin A per hundred grams was rated an insignificant source. From 20 to 99 units was considered fair; from 100 to 999 units, good; from 1,000 to 10,000 units, excellent, and over 10,000 units, outside the class of an ordinary food. The limiting value for excellent was selected on the basis of the richest food sources. The lower limit for good was then chosen so that foods in this class would supply a significant proportion of the day's requirement. These descriptive terms should not be interpreted too categorically, for it is necessary to take into account the quantity of the food usually eaten.

In considering quantitative values it is essential to remember that a single value allotted to any food must be treated as a selected representative value within the range of variation of different samples. The more the vitamin activity of foods is studied the more patent it becomes that in most cases there exists a wide variation from sample to sample even when the effective factors are controlled as far as possible.

In foods of plant origin there seems to be a direct parallel between greenness—chlorophyll content—and vitamin A activity. Although it is known that plant tissue owes its vitamin A activity to the presence of carotene, the relation, if any, between the presence of chlorophyll and the formation of carotene is not yet clear.

Thin green leaves are among the best sources of vitamin A, many of the fresh leaves assaying well over 1,000 units (Sherman) per hundred grams and some even as high as from 20,000 to 30,000 units. The parallelism between vitamin A content and greenness is made more apparent by a study of the relative vitamin A content of green and of bleached leaves from the same plant. The outer green leaves of iceberg lettuce, which are usually removed before the head is offered to the consumer, have been shown, for instance, to be from thirty to forty times as rich in vitamin A as the inner "bleached" leaves, which make up the head. The outer green leaves of a head of young cabbage are much higher in vitamin A content than the inner white leaves.

Other types of green vegetables having a high vitamin A potency include green seeds and seed foods such as peas and green snap beans, fleshy vegetables such as green peppers, and green stems such as asparagus, broccoli stalks and green celery, although these products are not as rich as the green leaves. Here again there is loss of vitamin A value with loss in green color, since bleached celery stalks contain little, if any, of the vitamin.

In many plant products vitamin A activity may be indicated by the yellow color of the carotene. Of the vegetables the most notable examples are carrots and sweet potatoes, both of which are excellent sources. A yellow color is especially valuable in detecting fruits that contain vitamin A, such as apricots, yellow peaches and bananas. When there is a yellow and a white variety of a given species, the yellow variety invariably has a higher vitamin A value than the so-called white variety. For example, yellow peaches and yellow tomatoes are good sources, whereas the corresponding white varieties are fair or insignificant sources. A yellow color cannot be taken as an infallible guide to vitamin A activity, however, since there are yellow plant pigments other than carotene.

Seeds such as the cereal grains, nuts and legumes are, with the exception of those having considerable green and yellow color, poor sources of vitamin A. Yellow corn—corn with a yellow endosperm—is the most important vitamin A food in this group.

A red color is sometimes mentioned as a guide to vitamin A content. It is true that such foods as red tomatoes and red peppers may be good sources. In these particular cases, however, it must be recalled that these products were green before they were red. On the whole, the association of a red color with high vitamin A content is probably more fortuitous than significant and should not be taken as anything more than indicative.

Thus a green or yellow color in foods of plant origin has come to be associated with high vitamin A activity, a rich green color indicating perhaps a relatively higher value than a yellow color.

Of the foods of animal origin, eggs, whole milk and milk products are the important sources, eggs having a rating of excellent, whole milk good and milk products of good or excellent, according to the proportion of milk fat they contain. Milk, however, since it is consumed in fairly large quantities, may be a more important source than its rating on the basis of units of vitamin A per hundred grams would imply.

Normally the cow derives her vitamin A entirely from plant material and the hen largely from the same source. Both carotene and vitamin A are secreted in milk and eggs, although the proportion of carotene to vitamin A is usually small.

The animal body does not seem to be capable of synthesizing vitamin A or the precursor carotene but must depend on its food for the needed supply. It is only reasonable to expect, therefore, that the vitamin A activity of such products as milk and eggs would vary according to the quantity of vitamin A in the diet of the respective species. There is ample evidence indicating that these products may vary between wide limits. This variability is not as great, however, as might be the case if the body were not capable of storing large quantities of vitamin A which may be released gradually during periods of low vitamin A intake, such as the period of winter feeding. That there is a gradually diminishing output of vitamin A during such periods rather than a sharp drop has been shown by the studies made by Fraps and his co-workers.[64] On the other hand, when the intake is increased there is a sharp rise in output unless the body store has been unduly depleted.

The total quantity of vitamin A put into the egg or the milk is small compared with the total quantity ingested by the respective species. There also seems to be a maximum value beyond which the vitamin A potency of milk, at least, cannot be raised.[65] Under similar feeding conditions the butter fat from different breeds of cows shows the same vitamin A activity, although the ratio of carotene to vitamin A may vary from breed to breed.[66]

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[64] Fraps, G. S.; Copeland, O. C., and Treichler, R.: The Vitamin A Requirements of Dairy Cows, Bull. 495, Texas Agricultural Experiment Station, 1934. Sherwood, R. M., and Fraps, G. S.: The Amount of Vitamin A Potency Required by Hens for Egg Production, Bull. 493, Texas Agricultural Experiment Station, 1934.

The relation between the color of the egg and the vitamin A content is often questioned, color referring to the yolk and not to the shell, since the latter is a breed characteristic and has nothing to do with nutritive value. In eggs available on the market at present, the color of the yolk is of little value as a basis for the judging of vitamin A potency. If the hen has derived her vitamin A supply from green feed or yellow corn, the yolks of the eggs will be deep yellow and rich in vitamin A. On the other hand, if the diet of the hen has contained cod liver oil and the hen has not had access to green or other pigmented food, the egg yolks may be light in color though rich in vitamin A.

Variability in the vitamin A content of milk and eggs from the standpoint of food value is perhaps not as serious a problem now as it might have been, since under present day feeding practices there is not such a wide seasonal variation in the vitamin A content of the diet of dairy cows and of poultry as existed formerly. Liver might also be included in a categorical list of foods rich in vitamin A, since it is one of the richest food sources. As Sherman [67] has pointed out, however, this product can hardly be considered an important source since its production is conditioned by the demand for other meat and therefore could not be increased to supply the quantity needed to make it a regular constituent of the diet.

A consideration of the importance of various untreated foods is a small part of the story of the food sources of any vitamin. A natural food may be a perfectly good source of a vitamin and yet before it comes to the table be subjected to such treatment that little or none of this factor is left. Vitamin A is less likely, perhaps, to be lost or inactivated during procedures of manufacture and preparation at home than some of the other vitamins, since it is fairly stable to heat and not appreciably soluble in water. It is destroyed, however, by oxidation, and foods that are heated for long periods show appreciable loss of vitamin A potency. It is not affected at the temperature of boiling water, so that foods cooked by boiling retain their vitamin A potency well. Canned foods have practically the same vitamin A value as the corresponding fresh foods. Canned products that have been stored for as long as from nine months to a year and in some cases for even longer periods still retain a large portion of their vitamin A potency.

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[65] Watson, S. J.; Bishop, Gerald, and Drummond, J. C.: The Relation of the Colour and Vitamin A Content of Butter to the Nature of the Ration Fed: I. Influence of the Ration on the Yellow Colour of the Butter, Biochem. J. **28**: 1076 (no. 3) 1934.

[66] Wilbur, J. W.; Hilton, J. H., and Hauge, S. M.: The Vitamin A Activity of Butter Produced by Guernsey and Ayrshire Cows, J. Dairy Sc. **16**: 153, 1933. Moore, Thomas: Vitamin A and Carotene: IX. Notes on the Conversion of Carotene to Vitamin A in the Cow, Biochem. J. **26**: 1 (no. 1) 1932. Booth, R. G.; Kon, S. K., and Gillam, A. E.: The Relative Biological Efficiencies of the Vitamin A and Carotene of Butter, ibid. **28**: 2169 (no. 6) 1934. Watson, Bishop and Drummond.[65]

[67] Sherman, H. C.: Chemistry of Food and Nutrition, ed. 5, New York, The MacMillan Company, p. 366.

The storing of foods in the frozen state offers one of the best methods of retaining the maximum vitamin value. The vitamin A value of frozen foods is for all practical purposes the same as that of the fresh products; provided, of course, that this statement is construed to apply to the frozen product or the defrosted product immediately after defrosting, since there may be a rapid loss of vitamin A value if the defrosted food is allowed to stand.

Dried, or dehydrated, foods show considerable loss in vitamin A content due, undoubtedly, to oxidation of the vitamin during the drying process. Dried products may show a further loss during storage.

Most foods in their natural state that are susceptible of storage for any length of time may be stored as long as from nine months to a year without serious loss of vitamin A value, and any loss taking place is very gradual.

CHAPTER V

VITAMIN A REQUIREMENTS AND PRACTICAL RECOMMENDATIONS FOR VITAMIN A INTAKE

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The estimation of vitamin A requirements necessitates the measurement of some physical or chemical criteria dependent on vitamin A intake. The known specific criteria which characterize a vitamin A deficiency in the animal organism have been summarized by Bessey and Wolbach.[1] Of the several recognized signs of vitamin A deficiency, it now appears that hemeralopia, or partial night blindness, is the earliest to appear. Fortunately it is a condition which lends itself readily to physical measurement. The amount of vitamin A that will just prevent hemeralopia probably represents something a little less than the minimum requirement. Certainly the vitamin A intake should not fall below the level that will be sufficient to prevent night blindness. A liberal margin of safety should be allowed for physiologic variations and for enabling the organism to lay by a store of this vitamin against such emergencies as illnesses attended with low or restricted intake of food. Still more liberal amounts should be provided for growing children, for persons recovering from wasting diseases and for pregnant and lactating women.

As a result of observations on the widespread and profound cytologic changes associated with vitamin A deficiency, Wolbach[2] has suggested that vitamin A is a structural material which may be solely concerned in maintaining an apparatus within cells and not in the chemical processes proper for which the apparatus is necessary. If such is the case, it would seem logical

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From the Bureau of Home Economics, U. S. Department of Agriculture.

[1] Bessey, Otto, and Wolbach, S. B.: The Physiology and Pathology of Vitamin A, J. A. M. A., to be published.

[2] Wolbach, S. B.: Vitamin Deficiency Experimentation as a Research Method in Biology, Science **86**: 569-576 (Dec. 24) 1937.

that vitamin A requirements should bear a definite relationship to the body weight rather than to energy metabolism, and in some of the data reviewed herein this deduction is strongly supported. A rough approximation of vitamin A requirements also would be obtained through a correlation of dietary surveys with the incidence of night blindness for large cross-sections of the population. Dietary surveys, resolved into terms of vitamin A intake, in conjunction with surveys of the incidence of night blindness made on the same group of persons, should furnish practical confirmation of the experimentally determined estimates of vitamin A requirements on relatively few rigidly controlled subjects.

A review of the work along these two lines of approach will reveal that only a feeble beginning has been made toward establishing a precise estimate of the vitamin A requirements of human beings and of factors which affect these requirements. It is not to be implied that measurements of dark adaptation are all that is to be desired as evidence of adequacy or inadequacy of vitamin A intake; they probably represent the clearest evidence that exists for the present. Nor should it be assumed that the instruments and means available for such measurements are the last word in either simplicity or precision.

FACTORS AFFECTING VITAMIN A REQUIREMENTS

It is quite generally conceded that the most careful control of experimental laboratory animals has not been rewarded by uniformity of response to graded intakes of vitamin A. This being the case with so well standardized an animal as the rat has now come to be, one may logically expect even greater physiologic variation in the vitamin A requirements of human beings. The only practical solution to the problem is to allow a generous margin of safety over any estimate based on studies of human subjects necessarily limited in number. Experimentation on the human vitamin A requirements needs to be conducted over a considerable period of time in order to obtain a fair estimate for individual subjects.

Certain physiologic conditions have been reported[3] to alter the utilization of vitamin A and its carotene precursors; for example: 1. Bile is essential for the utilization of carotene but apparently not for the utilization of vitamin A; also, if bile is shunted from the small intestine to the colon, the utilization of carotene is prevented. 2. Liquid petrolatum does not interfere markedly with absorption of vitamin A from the intestine but does inhibit the absorption of carotene. 3. The composition of the diet, other than its vitamin A content, has been reported to affect the absorption and utilization of both vitamin A and provitamin A.

Wilson, Das Gupta and Ahmad[3] studied the influence of the fat content of the diet in relation to the absorption of the carotene of raw carrots or of cooked spinach in an adult human subject. They found that only about 50 per cent of the carotene was absorbed when the diet was devoid of fat, whereas from 80 to 90 per cent was absorbed when the diet contained moderate amounts of fat. Basu[3] conducted similar but more extended experiments on the absorption of vitamin A and of carotene by white rats and concluded that different oils varied in their effect on absorption and utilization of vitamin A and of carotene according to the degree of unsaturation of the oil. Utilization was better with the more unsaturated oils. Basu also suggested that the essential unsaturated fatty acids may be linked with the utilization of the vitamin A activity of the food rather than that absence of the fatty acids in themselves represents a distinct dietary deficiency.

Friderichsen and Edmund[4] have reported striking differences in the utilization of the vitamin A of fish liver oils and the carotene of carrots or spinach by young children under 2 years of age. The dried spinach induced discernible improvement in dark adaptation with about one tenth the dosage (in terms of international units) required in the form of fish liver oils. The response with dried spinach was also greatly prolonged over that induced by fish liver oils. Relatively large quantities of carrot juice, mashed carrots or β-carotene often proved to be ineffective in restoring normal dark adaptation in young children who showed distinct evidence of subnormal dark adaptation.

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[3] Utilization of Vitamin A and Carotene, editorial, J. A. M. A. **109:** 1045 (Sept. 25) 1937. Basu, N. K.: Vitamin A and Fat Metabolism, Vitaminforschung **6:** 106-110 (April) 1937. Wilson, H. E. C.; Das Gupta, S. M., and Ahmad, Bashir: Studies on the Absorption of Carotene and Vitamin A in the Human Subject, Indian J. M. Research **24:** 807-811 (Jan.) 1937.

[4] Friderichsen, C., and Edmund, Carsten: Studies of Hypovitaminosis A: II. A New Method for Testing the Resorption of Vitamin A from Medicaments, Am. J. Dis. Child. **53:** 89-109 (Jan.) 1937; III. Clinical Experiments in the Vitamin A Balance in Children After Various Diets, ibid. **53:** 1179-1201 (March) 1937.

It must be concluded that our knowledge of the factors which affect the utilization of the vitamin A activity of foods and of the physiologic conditions which may alter these is for the present very limited. Since it is customary to evaluate the vitamin A content of diets on the basis of biologic assay values as determined with laboratory animals (rats) and without regard to the particular vitamin A or carotene sources, the practical solution lies in providing for liberal amounts of vitamin A in the human diet.

VITAMIN A REQUIREMENTS FOR THE PREVENTION OF NIGHT BLINDNESS

The vitamin A units, as expressed throughout this article, refer to U. S. P. or international unit values in accordance with the requirements of the Council on Pharmacy and Chemistry [5] for labeling vitamin A and vitamin D preparations.

Investigators have applied various means for the measurement of the rate of dark adaptation to the problem of estimating vitamin A requirements of human beings. These several methods, however, involve a principle which is common to all; namely, the measurement of the power of the eyes to adapt themselves to dim illumination. Some subjects are maximally adapted to the dark, and the light threshold measured immediately thereafter is taken as a criterion of dark adaptation; in other instances, the subjects are adapted to a moderately bright light (with or without preliminary dark adaptation) and measurements of light thresholds are made at intervals in darkness following this. Hecht [6] has recently published a summary of the chemistry and kinetics of dark adaptation and has outlined the types of dark adaptation responses which follow adaptation to light of different intensities.

Friderichsen and Edmund [4] made a study of 106 children under 2 years of age. The children were adapted to the dark for thirty minutes and then held at a distance of 10 cm. from a lamp, the apparent brightness of which could be varied by suitably graded filters for measurement of the light threshold. A child's positive response to light was indicated by certain characteristic reflex movements of the child's brow and eyelids. Although these workers developed a method which would seem highly promising if applied to the problem of the daily requirement of very young children for vitamin A, their work to date has been limited to a study of the utilization of carotene and vitamin A from different sources. Their observations have already been discussed.

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[5] New and Nonofficial Remedies, Chicago, American Medical Association, 1937, p. 448.

[6] Hecht, Selig: Rods, Cones and the Chemical Basis of Vision, Physiol. Rev. **17**: 239-290 (April) 1937.

Jeans and his co-workers [7] have reported on the use of the Birch-Hirschfeld instrument, later displaced in their work by the "biophotometer," for the measurement of dark adaptation as a practical clinical method for detecting vitamin A deficiency. Although some criticism of the reliability and reproducibility of measurements made with the biophotometer has been reported [8] in a survey study of school children, other investigators [9] have found the biophotometer and the Birch-Hirschfeld instruments satisfactory and useful for the purpose of detecting moderate degrees of night blindness. Feldman,[10] Edmund [11] and Hecht [6] describe the application of other means of measuring dark adaptation in which different sets of optical principles have been employed.

It would seem that the present difficulties surrounding any and all methods of measuring dark adaptation are at least as great as those involved in measuring basal metabolic rates. The method of Friderichsen and Edmund is limited to children so young that voluntary actions are not a complicating feature, but these authors mention that the tests are not easy to make but require great care, experience and knowledge of the reactions of small children. The methods adapted to older children and adults require the willing cooperation of the subject and an intelligence level corresponding to at least that of an average 6 year old child.

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[7] (*a*) Jeans, P. C., and Zentmire, Zelma: A Clinical Method for Determining Moderate Degrees of Vitamin A Deficiency, J. A. M. A. **102:** 892-895 (March 24) 1934; (*b*) The Prevalence of Vitamin A Deficiency Among Iowa Children, ibid. **106:** 996-997 (March 21) 1936. (*c*) Jeans, P. C.; Blanchard, Evelyn, and Zentmire, Zelma: Dark Adaptation and Vitamin A, J. A. M. A. **108:** 451-458 (Feb. 6) 1937.

[8] Palmer, C. E., and Blumberg, Harold: The Use of a Dark Adaptation Technic (Biophotometer) in the Measurement of Vitamin A Deficiency in Children, Pub. Health Rep. **52:** 1403-1418 (Oct.) 1937.

[9] Jeghers, Harold: The Degree and Prevalence of Vitamin A Deficiency in Adults, J. A. M. A. **109:** 756-761 (Sept. 4) 1937. Maitra, M. K., and Harris, L. J.: Vitamin A Deficiency Among School Children in London and Cambridge, Lancet **2:** 1009-1014 (Oct. 30) 1937. Corlette, M. B.; Youmans, J. B.; Frank, Helen, and Corlette, Mildred G.: Photometric Studies of Visual Adaptation in Relation to Mild Vitamin A Deficiency in Adults, Am. J. M. Sc. **195:** 54-65 (Jan.) 1938.

[10] Feldman, J. B.: Instrument for Determining Course of Dark Adaptation and for Measuring Minimum Light Threshold, Arch. Ophth. **12:** 81-85 (July) 1934.

[11] Edmund, Carsten: Some Methods of Measuring Dark Vision, Acta. ophth. **3:** 153-169, 1925.

Observations by Jeans and his co-workers [7c] on two 11 year old boys indicate that 3,000 units of vitamin A daily met their requirements as judged by the biophotometer test and standards which they consider normal responses. This level of vitamin A presumably is suggested not as the minimal level which would maintain normal dark adaptation but as an entirely adequate amount which might be considerably above the minimal requirement.

Jeghers,[9] using the biophotometer test, has reported that the dark adaptation of a normal adult was not maintained at peak efficiency for more than about one week when the vitamin A intake was limited to 200 units a day, despite the fact that the subject had previously received massive daily doses of vitamin A for a period of three months. A normal adult (weighing 52 Kg.) subjected to a very similar dietary program and studied at the Bureau of Home Economics, U. S. Department of Agriculture,[12] but who had not taken vitamin A except for that available in an ordinary freely chosen diet, showed definitely impaired dark adaptation [13] only after a period of approximately one month. After the impaired dark adaptation became apparent, it progressed with amazing rapidity until arrested by the administration of vitamin A. Whether this great difference in depletion of vitamin A reserves of the two subjects mentioned is indicative of the magnitude of the physiologic variations to be expected can only be left for further experimentation to determine. It might be of interest also to mention that the experimental subject studied at the Bureau of Home Economics showed no evidence of keratinization of the epithelium of the cornea at the time when dark adaptation was slowest but did show a marked constriction of the field of vision when tested [14] by means of a perimeter. A level of from 18 to 20 units of vitamin

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[12] Booher, Lela E.; Callison, Elizabeth C., and Hewston, Elizabeth Unpublished data.

[13] As measured by the visual adaptometer, the optical arrangements for which are those used in dark adaptation studies of Hecht.[6]

[14] Tests made by Drs. Louise Sloan and Allen Wood, Wilmer Eye Clinic, Johns Hopkins University, Baltimore.

A (corresponding to about 6 micrograms of vitamin A) per kilogram of body weight resulted in very slow improvement in the dark adaptation of this subject, whereas from 30 to 35 units per kilogram of body weight soon restored and maintained maximum dark adaptation. This level would amount to approximately 2,100 to 2,450 units of vitamin A for an adult of 70 Kg.

Edmund and Clemmesen[15] have recently reported a study of the vitamin A requirements of young adult men as carried out at the state prison in Nyborg (Denmark). Measurements of visual adaptation were made on twenty-eight young male prisoners in the summer and autumn of 1936, during which interval all the subjects received the fixed prison allotment of food. Following this the subjects were divided into two groups, one of which continued to consume this regular fare while the other group received in addition a half liter (about one pint) of whole fluid milk daily for a six month (autumn and winter) experimental period. The fourteen subjects receiving the fixed prison allowance, which contained an average of 1,225 units of vitamin A daily, showed seasonal oscillations in their powers of visual distinction (at different standard intensities of illumination) and occasional dysadaptation in some few cases. The subjects receiving the fixed prison allowance plus the milk supplement, the total food containing an average of 1,370 units of vitamin A daily, showed normal visual adaptation at all seasons. The vitamin A values assigned to the food items in the fixed prison allowance, being average values, do not disclose the natural seasonal variations in the vitamin A content of the food items. Actually the vitamin A intake of the control group would be a little more than 1,225 units during the summer months and a little less than 1,225 units during the winter months. It therefore required a year-round average of 1,370 units of vitamin A daily to provide an overall coverage for seasonal variations in the vitamin A values of the food in order to insure normal visual adaptations at all seasons. It was concluded, therefore, that about 1,400 units of vitamin A daily for an adult weighing from 68 to 69 Kg. would suffice for the support of normal visual adaptation.

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[15] Edmund, Carsten, and Clemmesen, S.: On Deficiency of A Vitamin and Visual Adaptation: II, translated by Mrs. Clara Packness, London, Oxford University Press, 1937.

According to Guilbert, Miller and Hughes,[16] a level of vitamin A or carotene intake which is just sufficient to prevent gross signs of hemeralopia suffices also for excellent gains in weight and for maintenance of a thrifty physical appearance of animals for indefinite periods, although the storage of the vitamin in the body may be very meager indeed. These workers report that several species of animals (cattle, sheep and swine) differing widely in body weight showed almost identical requirements for vitamin A or carotene per kilogram of body weight for the prevention of hemeralopia. It was suggested that the minimal requirements of other vertebrate species, including the human being, might bear the same quantitative relationship to body weight. A daily intake of approximately 40 to 50 units [17] in the form of carotene ("25 to 30 micrograms of carotene") or about 20 to 30 units [17] of vitamin A ("6 to 8 micrograms of vitamin A") per kilogram of body weight was found to be sufficient for the prevention of night blindness in these three species of animals. If the same requirement per kilogram of body weight holds for the human species, a man weighing 70 Kg. would require daily about 1,400 to 2,000 units of vitamin A or, roughly, double this number of units in the form of carotene as a "physiologic minimum."

Experimentation with animals has shown [16] quite conclusively that liberal allowances of vitamin A over and above the daily requirement which will support normal dark adaptation, an average growth rate and an outward thrifty physical condition are essential for maintaining bodily reserves of vitamin A and for the successful bearing and rearing of young. Experiments with animals have shown [18] that a liberal intake of vitamin A is also conducive to longevity and to prolonging the prime of life.

On the basis of the limited number of quantitative studies on record, it would appear that the vitamin A requirements for the prevention of partial night blindness in adults was of the order of from 25 to 30 units per kilogram of body weight daily, or a total of approximately 1,400 to 2,000 units daily for an adult weighing 70 Kg. It would also seem that the daily intake of vitamin A per kilogram of body weight of adults required for the prevention of night blindness might be very nearly a constant for vertebrate species of animals generally. However, further quantitative studies on the vitamin A requirements of children are necessary in order that children's requirements may be interpreted in relation to adult requirement. Many more studies will also be needed before the magnitude of physiologic variation in vitamin A requirement can be estimated.

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[16] Guilbert, H. R.; Miller, R. F., and Hughes, E. H.: The Minimum Vitamin A and Carotene Requirement of Cattle, Sheep and Swine, J. Nutrition **13**: 543-564 (May) 1937.

[17] The U. S. P. or international units have been calculated by the author from the data as given by the investigators, 0.6 microgram of carotene and 0.3 microgram of vitamin A being considered equivalent to one U. S. P. or international unit. The quotations give the quantities in the terms used by the investigators.

[18] Batchelder, Esther L.: Nutritional Significance of Vitamin A Throughout the Life Cycle, Am. J. Physiol. **109**: 430-435 (Sept.) 1934.

ANALYSIS OF DIETARY SURVEYS IN TERMS OF VITAMIN A INTAKE

Dietary surveys resolved into terms of the essential food constituents, when compared with dietary requirements for the same food essentials, furnish a general index of the probable nutritive status of different population groups. In addition, these surveys point the way to specific means for dietary improvements.

The practical problem of food selection is influenced by several factors operating simultaneously and including family income or, more precisely, the amount of money spent for food, degree of urbanization, individual preference and application of available knowledge of foods and nutrition. In general, the classification of dietaries according to the amount of money spent for food per adult capita shows a distinct gradation in the qualities of the diets. Within any one of these classes further differences are observed according to the degrees of urbanization. With specific reference to vitamin A, it is quite obvious that foods rich in vitamin A are not the least expensive of the ordinary food items. The families living in cities or on farms in each region tend to be provided with more liberal allowances of vitamin A than families living in villages. The most probable explanation of this is that the city dwellers have access to good markets; the farm families can provide themselves with milk, butter and eggs as well as garden produce from their own farms, while the village folk, generally, share less of either of these advantages.

The average adult vitamin A intake daily was computed from a dietary survey made during the year 1935-1936 in different regions of the United States. At present data for the average vitamin A values of the common foods determined directly in terms of U. S. P. or international units are insufficient for the purpose of evaluating the total vitamin A value of ordinary mixed diets. The vitamin A values of most of the common food items are, however, available in terms of Sherman units of vitamin A. Information furnished by several laboratories of recognized standing and having extended experience with the U. S. P. method of vitamin A assay indicate, from the growth rates of vitamin A assay rats which were fed the standard U. S. P. reference cod liver oil, that the value in Sherman units divided by a factor of from 1.5 to 2 is approximately equivalent to the value in U. S. P. or international units.[19] The data for the dietary survey reported in the accompanying table were originally computed in terms of Sherman units of vitamin A. For purposes of correlation with the rest of the observations reported here these values have been divided by 2 as probably representing a fair approximation in terms of U. S. P. or international units of vitamin A.

The data in this table show quite strikingly how the average vitamin A intake varies with the amount of money available for food and for any one "food-spending group" with the degree of urbanization. A careful examination of the specific food items actually consumed by these different population groups also showed the need for popular education on the subject of foods and nutrition. While some families had too little money to buy a fully adequate food supply regardless of how carefully they might select it, still a large proportion now existing on poor diets would be able to obtain a fully adequate diet if they had applied the present knowledge of nutrition to the problem of the best selection of food.

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[19] Dr. E. M. Nelson, Food and Drug Administration, U. S. D. A., states in a personal communication: "When U. S. P. units of vitamins A and D based on international units for these vitamins came into use it seemed desirable to acquaint investigators in this country with the approximate relative values of international units and units which had previously been used in this country. On the basis of data obtained in assay of the U. S. P. reference cod liver oil against the international standard for vitamin A, it was possible to make an estimation of these relative values. The U. S. P. Vitamin Advisory Board issued a statement in which it was stated that 1 U. S. P. X Sherman or American Drug Manufacturers' Association vitamin A unit was approximately equivalent to 1.4 international or U. S. P. X (Revised 1934) vitamin A units. It was stated: 'These conversion factors should not be considered as having any official recognition.' Since that time it has become apparent that the conversion factor proposed at that time was inaccurate. Most of the assays that have come to my attention since that time show that 1 international unit of vitamin A will promote a growth rate in excess of 3 Gm. per week during the assay period in the U. S. P. method, indicating that a given product would contain fewer international than Sherman units. Since manufacturers of pharmaceutical preparations changed their assay procedure so as to determine vitamin A potency directly in terms of U. S. P. X (Revised 1934), or international, units this inaccuracy has been of no particular moment in the labeling of such products, and for this reason the U. S. Pharmacopeia had not concerned itself further with conversion factors of different vitamin A units."

It is apparent that many members of families in the lower income groups probably are on the borderline of a recognizable vitamin A deficiency. Because each of these group averages embraces rather wide individual variations in vitamin A intake, it is to be expected that a fair proportion of persons in the lowest income groups actually receive less than the "physiologic minimum" requirement of vitamin A. This deduction is amply borne out by other surveys on the incidence of partial night blindness.

Vitamin A Value of Diets of Families Not on Relief, 1935-1936 (in U. S. P. or International Units) *

Region and Degree of Urbanization	Average Units per Adult per Day† for Families Spending Weekly per Head for Food				
	$1.25- $1.87		$2.50- $3.12	$3.13- $3.74	$3.75- $4.37
City ‡					
North Atlantic	2,200	2,800	3,400	4,200	5,700
East North Central	2,600	2,500	3,500	3,000	5,000
East South Central	3,600	4,600	6,200	.....	.....
Pacific	3,300	4,000	4,500	5,400	5,700
Village §					
North Central	1,500		2,900		
Southeast	2,600		4,100		6,000
Pacific	2,500		.....		3,700
Farm ‖					
North Central	3,000		3,900		4,200
Southeast	3,600		5,400		7,200
Pacific	3,600		5,000		

* Data furnished by Dr. Hazel K. Stiebeling, Senior Food Economist, Bureau of Home Economics, U. S. Department of Agriculture.

† The following scale of family coefficients was employed in evaluating the vitamin A intake for families of different types in terms of an adult:

Child under 4 years	0.75
Boy 4-6; girl 4-7 years	0.75
Boy 7-8; girl 8-10 years	0.90
Boy 9-10; girl 11-13 years	0.90
Adult (man or woman)	1.00

These coefficients are essentially the same as the factors used to evaluate the adult intake of protein.

‡ Families of employed workers. Field work conducted by the U. S. Bureau of Labor Statistics.

§ All occupational groups.

‖ Farm operators. Farm-furnished food valued at farm prices.

Jeans and Zentmire[7b] tested 404 country and village school children of Iowa from 6 to 15 years of age and found latent night blindness in 26 per cent of the country children and in 53 per cent of the village children. The highest incidence of night blindness was found in families of the lowest income classes.

Maitra and Harris [9] found definitely subnormal dark adaptation among elementary school children (from 8 to 14 years of age) in London and Cambridge to the extent of about 30 per cent of the 193 children examined. These investigators also found a tendency toward higher incidence of hemeralopia among the younger children of the group.

The vitamin A reserves of new-born infants have been reported [20] to be very low, the livers of infants being found to contain only about 14 to 17 units of vitamin A per gram in contrast to an average of 220 units of vitamin A per gram reported [21] for the livers of healthy adults. The vitamin A reserves of a well fed infant rise very rapidly during the first few months of life. The premature and artificially fed infant needs special attention with regard to the vitamin A supply of the food.

A very high incidence of "dysadaptation" has been found among pregnant women. Edmund and Clemmesen [22] report that 50 per cent of the pregnant women admitted to a Copenhagen municipal hospital showed signs of subnormal visual adaptation.

PRACTICAL RECOMMENDATIONS FOR VITAMIN A INTAKE FOR PERSONS OF DIFFERENT AGES

The physiologic minimum requirement of vitamin A for a man weighing 70 Kg. would appear to be on the order of from 1,400 to 2,000 units of vitamin A daily. Provision for a supply of vitamin A which furnishes barely enough to prevent measurable signs of deficiency is of course unsound and in serious opposition to the best interests of the health and welfare of subjects at any age. A 50 per cent margin of safety, providing 3,000 units of vitmain A daily for an adult weighing 70 Kg., would seem to be none too liberal in view of the facts that there may be considerable physiologic variation in the requirement for vitamin A, that at least a moderate storage probably is highly desirable and that there is some question about the relative utilization of the carotene sources of this vitamin.

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[20] Ellison, J. B., and Moore, Thomas: Vitamin A Reserves of the Human Infant and Child in Health and Disease, J. Soc. Chem. Ind. **55:** 236 (March 20) 1936.

[21] Moore, Thomas: The Vitamin A Reserves of the Adult Human in Health and Disease, J. Soc. Chem. Ind. **55:** 235-236 (March 20) 1936.

[22] Edmund, Carsten, and Clemmesen, S.: On a Deficiency of A Vitamin and Visual Adaptation, London, Oxford University Press, 1936.

The Technical Commission for the Study of Nutrition of the Health Organisation of the League of Nations recommends [23] an allowance of from 2,000 to 4,000 units daily for an adult as fully adequate on the basis of an intake of 500 cc. of whole fluid milk, one egg, 25 Gm. of butter and a medium serving of a green, leafy vegetable daily. This recommendation of from 2,000 to 4,000 units of vitamin A was suggested in view of the range of from 1,400 to 3,000 units suggested by different investigators as a minimum requirement, the latter range being partly or mainly accounted for, it was supposed, by differences in the vitamin A content assigned to common foods by various workers.

The diets of infants and young children usually contain more milk than the diets of adults, and their diets are frequently supplemented with some kind of fish liver oil, so that in comparison with well nourished adults the well fed infant probably receives a considerably larger amount of vitamin A per kilogram of body weight. Any extra needs associated with rapid rates of growth would thus be taken care of as well as reasonable provision for building up a bodily reserve of vitamin A. It would seem important in the case of both premature infants and artificially fed infants to pay even more particular attention to the inclusion of liberal amounts of the so-called protective foods in order to permit them to build satisfactory bodily reserves of vitamin A and to supply adequate amounts of minerals and other vitamins as well.

The Technical Commission of the Health Committee of the League of Nations has recommended [24] that all children between the ages of 2 and 14 years be provided with about 1 quart of milk daily, in addition to an egg, servings of green, leafy vegetables and butter suited to the size of the child, and 3 Gm. of cod liver oil daily as the main sources of protective foods rich in vitamin A. These recommendations probably would provide for at least 6,000 to 8,000 units of vitamin

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[23] Geneva Bulletin of the Health Organisation, League of Nations, vol. VII, June 1938.

[24] League of Nations, Health Organisation: Report on the Physiological Bases of Nutrition, Geneva, 1935.

A daily for growing children. For a more specific account of these recommendations the reader is referred to the publication cited.

A very effective means of dealing with the problem of supplying an adequate vitamin A intake for children of school age would be to serve carefully planned school lunches which would furnish liberal amounts of the so-called protective foods. Rose and her co-workers [25] and Hann and Stiebeling [26] have shown in very practical terms how school lunches may be planned to furnish more than 50 per cent of the probable vitamin A requirements of children of nursery and elementary school ages and to improve the diets of the children in other ways at one and the same time.

The Technical Commission of the Health Committee of the League of Nations recommends [24] a daily intake of at least 5,000 U. S. P. or international units of vitamin A for the pregnant and nursing woman. In terms of food items this may be translated as 1 quart of fresh, whole milk, one egg, 1 ounce of cheese, an average serving of a green, leafy vegetable and 1 teaspoonful of cod liver oil daily.

SUMMARY

The daily requirement for vitamin A just sufficient for the prevention of night blindness in a normal adult would appear to be on the order of from 20 to 30 U. S. P. units of this vitamin per kilogram of body weight, or a total of about 1,400 to 2,000 units daily for an adult weighing 70 Kg.

Allowing for a fair margin of safety and for the maintenance of a moderate storage of vitamin A in the body, a total of around 3,000 units of vitamin A daily is suggested for the normal adult. In view of other qualities of nutritional excellence provided by milk, butter, eggs and green leafy vegetables, these food items should be used in quantities which will provide a large proportion of this vitamin A allowance.

Provision of around 6,000 to 8,000 units of vitamin A daily for the growing child would presumably be adequate to take care of any extra needs associated with growth and development and to provide for a moderate bodily storage of vitamin A. A small supplement of some fish liver oil in addition to liberal quantities of whole milk, butter, eggs and green leafy vegetables is recommended for children in view of the excellence of these food items in vitamin A and other nutritive essentials important for the growing child. Particular attention should be paid to supplying the infant and even more especially the premature or artificially fed infant with liberal quantities of vitamin A, since infants are born with very meager stores of vitamin A in their livers.

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[25] Rose, Mary S.: Foundations of Nutrition, New York, Macmillan Company, 1938.

[26] Hann, Helen N., and Stiebeling, Hazel K.: Food Consumption of Children at the National Child Research Center, U. S. D. A. Circular, to be published.

The recommended allowance of vitamin A for pregnant and nursing women has been set at around 5,000 units or more daily, with a further suggestion that this allowance be supplied mainly by liberal amounts of milk, butter, cheese, eggs, green leafy vegetables and some small addition of fish liver oil.

CHAPTER VI

THE COMPONENTS OF THE VITAMIN B COMPLEX

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The purpose of this paper is to provide the reader with a picture of what is usually called the "vitamin B complex" and to explain the nomenclature that has been used to designate individual members of the complex. It is apparent to any one who reads a number of papers on this subject that it is difficult to follow the terminology. Confusion has resulted in the past largely because different terms have been applied to newly discovered factors. In addition, specific terms have been coined before the existence of separate entities has been definitely established. Now, however, through chemical isolation and synthesis, through more extensive biologic researches and through clarification of designations, the veil of confusion is lifting and four of the so-called B vitamins are quite clearly discerned. Brilliant researches have elucidated the chemical nature of vitamin B_1, riboflavin and vitamin B_6, and nicotinic acid has been found to be effective in the cure of human pellagra. Three of these factors will be discussed in subsequent papers.

The members of the B complex may be designated and described as follows:

Vitamin B_1, the antiberiberi vitamin that prevents beriberi in man and polyneuritis in animals.

Riboflavin, a compound necessary for growth in chicks and rats and for the prevention of cataract in rats. It is a component of an oxidation-reduction system of living cells.

P-P factor, a nutritional factor effective in the prevention of human pellagra, and recently shown to be nicotinic acid.

Filtrate factor, or antidermatitis factor, effective in the prevention of nutritional dermatosis in chicks.

Vitamin B_3, a factor necessary for rapid gains in weight and normal nutrition of pigeons.

Vitamin B_4, a factor for the prevention of a specific paralysis in rats and chicks.

From the Food and Drug Administration, U. S. Department ot Agriculture.

Vitamin B_5, a factor necessary for maintenance of weight in pigeons.

Vitamin B_6, or Factor I, a factor for the prevention of a nutritional dermatosis in rats.

Factor W, a factor necessary for growth of rats.

HISTORICAL DEVELOPMENT

Vitamin B_1 stands unique in that the present vitamin theory was first conceived and developed from studies on the antiberiberi factor and in that the class name was derived from early work on its chemical nature. The story of this vitamin goes back to the epoch making investigations on beriberi by a group of Dutch medical officers working in the Dutch East Indies. Among them were notably Eijkman,[1] who contributed the discovery that the disease could be produced experimentally in fowls, and Grijns,[2] who contributed the rather revolutionary theory that the disease was due to a nutritional deficiency and not to toxicity or infection, thereby laying the foundation for the present conception of vitamins. In 1911 Funk [3] published a series of papers dealing with the isolation from rice polishings of the substance active in the cure of beriberi. The following year this investigator [4] stated: "The deficient substances, which are of the nature of organic bases, we will call 'vitamines'; and we will speak of a beri-beri or scurvy vitamine, which means a substance preventing the special disease." This name put several nutritional deficiency diseases into a separate class and placed emphasis on them.

Concurrently with studies on beriberi, in attempts to ascertain the nature of all dietary essentials, Hopkins,[5] Osborne and Mendel,[6] and McCollum [7] provided evidence that certain previously unidentified factors were necessary for the growth of rats. McCollum [8] proposed the name "water-soluble B" for one of these factors and presented evidence indicating that water-soluble B and the beriberi vitamin were similar in nature. The terms "beriberi vitamine," "antineuritic vitamine" and "water-soluble B" were used until 1920, when Drummond [9] made the following recommendation, which met with general approval:

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[1] Eijkman, Christiaan: Eine Beri Beri—ähnliche Krankheit der Hühner, Virchows Arch. f. path. Anat. **148**: 523-532 (June) 1897.

[2] Dr. Gerrit Grijns' Researches on Vitamins, 1900-1911, Gorinchem, J. Noorduyn en Zoon N. V., 1935.

[3] Funk, Casimir: On the Chemical Nature of the Substance Which Cures Polyneuritis in Birds Induced by a Diet of Polished Rice, J. Physiol. **43**: 395-400 (Dec.) 1911.

[4] Funk, Casimir: The Etiology of the Deficiency Disease, J. State Med. **20**: [illegible] (May) 1912.

[5] Hopkins, F. G.: Feeding Experiments Illustrating the Importance of Accessory Factors in Normal Dietaries, J. Physiol. **44**: 425-460 (July) 1912.

[6] Osborne, T. B., and Mendel, L. B.: The Influence of Butterfat on Growth, J. Biol. Chem. **16**: 423-437 (Nov.) 1913.

[7] McCollum, E. V., and Davis, Marguerite: The Necessity of Certain Lipins in the Diet During Growth, J. Biol. Chem. **15**: 167-175 (July) 1913.

> The suggestion is now advanced that the final "e" be dropped, so that the resulting word Vitamin is acceptable under the standard scheme of nomenclature adopted by the Chemical Society, which permits a neutral substance of undefined composition to bear a name ending in "in." If this suggestion is adopted, it is recommended that the somewhat cumbrous nomenclature introduced by McCollum (Fat-soluble A, Water-soluble B), be dropped, and that the substances be spoken of as Vitamin A, B, C, etc. This simplified scheme should be quite sufficient until such time as the factors are isolated, and their true nature identified.

While there was not unanimity of opinion with respect to the identity of the water-soluble growth-promoting substance for the rat and the beriberi vitamin prior to 1920,[10] experimental evidence of the existence of a complex of important substances was developed largely after that date. In 1920 Emmett and Luros [11] reported the first investigation, which clearly indicated that water-soluble B and the antineuritic vitamin were not equally susceptible to destruction by heat and stated that they should be considered different factors. Though a number of other investigators contributed to these studies, several papers from the United States Public Health Service seemed to establish beyond reasonable doubt that vitamin B was not a single chemical entity. Goldberger and Tanner [12] announced that pellagra was due to a dietary deficiency and that among other products which would prevent the disease was brewers' yeast. They proposed the name "pellagra-preventive factor" or "P-P factor" for this dietary essential. Smith and Hendrick[13] found that yeast which had been autoclaved to destroy the antineuritic vitamin had a definite supplementing value in a diet for rats in which oats or Seidell's vitamin B picrate was the source of vitamin B. In a paper by Goldberger and his associates[14] on the pellagra preventives in relation to vitamin B, they stated: "In any event investigators using the rat-growth test must hereafter recognize and take due account of at least two essentials (B sensu stricto and P-P) where heretofore only one was considered." Goldberger and Lillie[15] produced a pellagra-like syndrome in rats using a ration to which had been added adequate amounts of the antineuritic vitamin. This condition could be prevented or cured by including small quantities of autoclaved yeast in the ration. In commenting on their observations they stated:

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[8] McCollum, E. V., and Kennedy, Cornelia: The Dietary Factors Operating in the Production of Polyneuritis, J. Biol. Chem. **24**: 491-502 (April) 1916.

[9] Drummond, J. C.: The Nomenclature of the So-Called Accessory Food Factors (Vitamins), Biochem. J. **14**: 660 (Oct.) 1920.

[10] Mitchell, H. H.: On the Identity of the Water-Soluble Growth-Promoting Vitamin and the Antineuritic Vitamin, J. Biol. Chem. **40**: 399-413 (Dec.) 1919.

[11] Emmett, A. D., and Luros, G. O.: Water-Soluble Vitamins: I. Are the Antineuritic and the Growth-Promoting Water-Soluble Vitamins the Same? J. Biol. Chem. **43**: 265-286 (Aug.) 1920.

[12] Goldberger, Joseph, and Tanner, W. F.: A Study of the Pellagra-Preventive Action of Dried Beans, Casein, Dried Milk and Brewers' Yeast with a Consideration of the Essential Preventive Factors Involved, Pub. Health Rep. **40**: 58-80 (Jan.) 1925.

> . . . the possibility remote though it seems is not excluded that there may be in yeast more than one such thermostable factor which further study may succeed in differentiating.

As continued evidence of the multiplicity of vitamin B has appeared, confusion in nomenclature and identity of factors has also grown. A few important contributions stand out in this maze which tend to clarify the picture. In 1927 Jansen and Donath[16] announced the isolation of the antiberiberi vitamin. Subsequently the researches of Williams[17] elucidated the structure of the compound, and synthesis was accomplished.[18] In

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[13] Smith, M. I., and Hendrick, E. G.: Some Nutrition Experiments with Brewers' Yeast with Especial Reference to Its Value in Supplementing Certain Deficiencies in Experimental Rations, Pub. Health Rep. **41:** 201-207 (Feb. 5) 1926.

[14] Goldberger, Joseph; Wheeler, G. A.; Lillie, R. D., and Rogers, L. M.: A Further Study of Butter, Fresh Beef and Yeast as Pellagra-Preventives, with Consideration of the Relation of Factor P-P of Pellagra (and Blacktongue in Dogs) to Vitamin B, Pub. Health Rep. **41:** 297-318 (Feb. 19) 1926.

[15] Goldberger, Joseph, and Lillie, R. D.: A Note on an Experimental Pellagra-Like Condition in the Albino Rat, Pub. Health Rep. **41:** 1025-1029 (May 26) 1926.

[16] Jansen, B. C. P., and Donath, W. F.: Isolation of Anti-Beriberi Vitamin, Mededeel. v. d. dienst. volksgezondh. in Nederl.-Indië **16:** 186-199, 1927.

[17] Williams, R. R.: Structure of Vitamin B_1, J. Am. Chem. Soc. **58:** 1063-1064 (June) 1936.

[18] Cline, J. K.; Williams, R. W.; Ruehle, A. E., and Waterman, R. E.: Studies of Crystalline Vitamin B: XVI. Identification of the Pyrimidine Portion, J. Am. Chem. Soc. **59:** 530-533 (March) 1937.

1933 Kuhn, György and Wagner-Jauregg[19] isolated a yellow-green fluorescent compound from egg white which they called "ovoflavin" and which was shown to have growth-promoting properties for the rat.[20] This compound has also been synthesized.[21] Further matters of nomenclature relate primarily to the three factors (antipellagra, antiberiberi and flavin) which have just been described. The other factors can be discussed more conveniently if terms are explained first.

TERMINOLOGY

In 1927 the British Accessory Food Factors Committee[22] approved a system of nomenclature in which vitamin B was used for the complex, vitamin B_1 for the antineuritic vitamin, and vitamin B_2 for the more heat-stable fraction of the complex. As evidence for the existence of other factors developed they were designated as vitamin B_3, vitamin B_4, vitamin B_5 and vitamin B_6. In 1929 a committee on vitamin B nomenclature for the American Society of Biological Chemists[23] recommended that the term "vitamin B" be used to designate the more heat labile factor or antiberiberi vitamin and "vitamin G" be used to designate the more heat stable fraction of the complex. This system of nomenclature has been confined almost exclusively to American literature. The term "vitamin G" has been used to some extent since that time to refer to the pellagra-preventive factor, but this terminology has not been used by the United States Public Health Service. Terminology that is not in accord with either the British or the American system has also been used. When Kuhn demonstrated that his flavin had properties of a vitamin he called it "vitamin B_2." Vitamin B_2 is used extensively in this sense in the German literature. Development of biologic methods has also influenced the usage of terms. Bourquin and Sherman[24] proposed a method for the determination of vitamin G, using the rat as the experimental animal. This method has been used extensively to determine the vitamin G content of foods and pharmaceutical preparations. In 1933 Booher[25] concluded that the Bourquin-Sherman method for the vitamin G determination was a measure of flavin. In summary it may be stated that the antiberiberi vitamin has been called "vitamin B" and "vitamin B_1." "Vitamin B_2" and "vitamin G" have both been used to designate that part of the B complex, other than B_1, to be synonymous with flavin and to be synonymous with the pellagra-preventive factor. In recent publications dealing with these factors, authors have frequently found it necessary to explain the system of nomenclature that they have been using.

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[19] Kuhn, Richard; György, Paul, and Wagner-Jauregg, Theodor: Ueber eine neue Klasse von Natur-farbstoffen (vorläufige mitteilung), Ber. chem. Gesellsch. **66:** 317-320 (Feb.) 1933; Ueber Ovoflavin, den Farbstoff des Eiklars, ibid. **66:** 576-580 (April) 1933.

[20] György, Paul; Kuhn, Richard, and Wagner-Jauregg, Theodor: Das Vitamin B_2, Naturwissenschaften **21:** 560-561 (July) 1933.

[21] Kuhn, Richard; Reinemund, Karl, and Weygand, Friedrich: Synthese des Lumi-lactoflavins, Ber. chem. Gesellsch. **67:** 1460-1462 (Aug.) 1934. Karrer, P.; Salomon, H.; Schöpp, K.; Schlittler, E., and Fritzsche, H.: Ein neues Bestrahlungsprodukt des *Lactoflavins:* Lumichron, Helvet. chem. acta. **17:** [illegible] (Oct.) 1934; Synthese Lactoflavin-ähnlicher Verbindungen, ibid. **17:** 1165-1169 (Oct.) 1934.

[22] Nomenclature adopted by the Biochemical Society in 1927. Dutcher, R. A.: Vitamin B Terminology, Science **68:** 206-209 (Aug. 31) 1928. Note on the Nomenclature of the Vitamins of the B Complex: Vitamins, Brit. Research Council, 1932, p. 118.

[23] Dutcher, R. A.: Vitamin B Terminology, Science **69:** 671 (March 8) 1929.

The Committee on Vitamin Nomenclature made the following recommendations to the American Society of Biological Chemists and the American Institute of Nutrition, at their 1937 annual meetings:

1. That the vitamin which is recognized as a specific in the prevention or cure of beriberi in man and polyneuritis in experimental animals, and which has been identified as consisting of a 2 methyl 4 amino pyrimidine group and a 4 methyl 5 B hydroxy ethyl thiazole group joined by a methylene group through carbon atom 5 of the pyrimidine ring and the nitrogen atom of the thiazole ring, be designated as "B_1," and that the term "B" without a subscript be no longer used.

2. That the compound identified as "6, 7 dimethyl-9 (d 1' ribityl) isoalloxazine" be designated as "riboflavin," and that the terms "vitamin B_2" or "vitamin G" be no longer used.

3. That the term "pellagra-preventive factor" or "P-P factor" be used to designate the nutritional factor effective in the prevention of human pellagra. The use of the term "pellagra" shall be restricted to pellagra in man and shall not be used, even though qualified, in referring to similar syndromes in other animals.

These recommendations were approved by both societies but final adoption was held in abeyance until the reactions to these proposals of other interested groups could be ascertained. The term "riboflavin" had previously been adopted by the Council on Pharmacy and Chemistry.[26] The terms "vitamin B_1," "riboflavin" and "pellagra-preventive factor," as already defined, will be used in the remainder of this paper. Since the announcement of Elvehjem and co-workers that nicotinic acid cures blacktongue in dogs,[26a] extensive clinical studies have provided convincing evidence that nicotinic acid complies with the definition for "the pellagra-preventive factor" of Goldberger.

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[24] Bourquin, Anne, and Sherman, H. C.: Quantitative Determination of Vitamin G (B_2), J. Am. Chem. Soc. **53**: 3501-3505 (Sept.) 1931.

[25] Booher, Lela E.: The Concentration and Probable Chemical Nature of Vitamin G, J. Biol. Chem. **102**: 39-46 (Sept.) 1933; **107**: 591-597 (Nov.) 1934.

OTHER MEMBERS OF THE VITAMIN B COMPLEX

Evidence of the existence of additional members of the B complex has largely been obtained by separations effected by selective adsorption, differences in stability to heat in mediums containing varying proportions of acid and alkali, and the response of rats, pigeons and growing chicks fed various types of diets. Feeding experiments differ in so many important details that a satisfactory appraisal or correlation of closely related investigations is not possible. While other factors or preparations could be discussed, it seems desirable to limit this brief presentation to the following, which will be designated according to what appears to be the most popular usage.

VITAMIN B_3

Williams and Waterman[27] described vitamin B_3, although its existence was noted by Schaumann,[28] Cooper[29] and Emmett and McKim[30] in their work relating to the B requirements of the pigeon. Williams and Waterman found that pigeons fed a diet of polished rice supplemented with autoclaved yeast and a vitamin B_1 concentrate did not gain weight. The same diet supplemented with air-dried brewers' yeast or whole wheat permitted a satisfactory growth response.

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[26] Riboflavin, the Accepted Name for Vitamin B_2, J. A. M. A. **108:** 1340-1341 (April 17) 1937.

[26a] Elvehjem, C. A.; Madden, R. J.; Strong, F. M., and Woolley, D. W. Communication, J. Am. Chem. Soc. **59:** 1767 (Sept.) 1937.

[27] Williams, R. R., and Waterman, R. E.: The Composite Nature of Vitamin B, Proc. Soc. Exper. Biol. & Med. **25:** 1-3 (Oct.) 1927; The Tripartite Nature of Vitamin B, J. Biol. Chem. **78:** 311-322 (July) 1928.

[28] Schaumann, H.: Further Contributions to the Etiology of Beriberi, Tr. Soc. Trop. Med. & Hyg. **5:** 59-75 (Dec.) 1911.

[29] Cooper, E. A.: On the Protective and Curative Properties of Certain Foodstuffs Against Polyneuritis Induced in Birds by a Diet of Polished Rice, J. Hyg. **12:** 436-462 (Dec.) 1912.

[30] Emmett, A. D., and McKim, L. H.: The Value of the Yeast Vitamin Fraction as a Supplement to a Rice Diet, J. Biol. Chem. **32:** 409-419 (Dec.) 1917.

Williams and Eddy[31] proposed the name "vitamin B_3" for this growth-promoting factor. Vitamin B_3 deficiency is characterized by a decline in the body weight, which can be rapidly restored by the addition of proper sources of the vitamin to the diet, and there also follows an improvement in the condition of the feathers, appetite and general activity.

Concentrates[32] with vitamin B_3 activity have been prepared from liver by extraction with dilute alcohol and adsorption of the active material on fullers' earth. The factor is more heat labile[32] than vitamin B_1, for when yeast is treated with alkali before it is dried at temperatures ranging from 20 to 60 C., practically all vitamin B_3 activity is lost while vitamin B_1 is still retained. It is soluble in water and dilute alcohol. It is present in yeast, liver, whole grains and malt but not in malt extract.

The necessity for this factor has been demonstrated with the pigeon, and, while experimental evidence has been presented to support the view that it is required by the chick,[33] the validity of this evidence has been questioned.[34]

VITAMIN B_4

Vitamin B_4 is concerned with the prevention of a specific paralysis in rats and chicks. Reader[35] found that if rats were fed a vitamin B deficient diet supplemented with autoclaved marmite, polyneuritis developed. When these animals were treated with a B_1 concentrate,[36] the typical symptoms of polyneuritis were relieved, but they still exhibited a condition of malnutrition characterized by a hunched back, lack of coordination and red swollen paws. This condition could be cured by administering a concentrate from yeast treated in a manner designed to free it from vitamin B_1. She proposed the name vitamin B_3 for this new factor and later changed the designation to B_4.[37] Failure in attempts to repeat and confirm Reader's observations led certain British investigators[38] to doubt the existence of vitamin B_4 and to conclude that the characteristic symptoms were a chronic vitamin B_1 deficiency which could be cured by large doses of vitamin B_1. However, a group of Wisconsin investigators[39] confirmed and extended Reader's original observation with rats subsequent to a demonstration that this factor was required by the chick.[40]

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[31] Williams, R. R., and Eddy, W. H.: A Report, Carnegie Institution of Washington Year Book **27**: 378, June 1927-July 1928.

[32] Carter, C. W., and O'Brien, J. R.: Maintenance Nutrition in the Pigeon: Vitamin B_3 Concentrates, Biochem. J. **30**: 43-47 (Jan.) 1936.

[33] Eddy, W. H.; Gurin, Samuel, and Keresztesy, John: The Williams-Waterman Vitamin B_3, J. Biol. Chem. **87**: 729-740 (April) 1930.

[34] Kline, O. L.; Keenan, J. A.; Elvehjem, C. A., and Hart, E. B.: The Use of the Chick Vitamin B_1 and B_2 Studies, J. Biol. Chem. **99**: 295-307 (Dec.) 1932.

[35] Reader, Vera: A Second Thermolabile Water-Soluble Accessory Factor Necessary for the Nutrition of the Rat, Biochem. J. **23**: 689-694 (No. 4) 1929; Further Evidence for a Third Accessory "B" Factor, Biochem. J. **24**: 77-80 (No. 1) 1930.

[36] Kinnersley, H. W.; O'Brien, J. R., and Peters, R. A.: Crystalline Vitamin B_1, Biochem. J. **29**: 701-715 (March) 1935.

This factor has been concentrated from aqueous extracts of yeast[41] and defatted liver[39a] by adsorption on active charcoal in an acid medium and elution with ethyl alcohol. It is very soluble in water, insoluble in fat solvents and labile to autoclaving in alkaline mediums.

VITAMIN B_5

Carter, Kinnersley and Peters[42] described an alkali heat-stable, slightly water-soluble factor present in yeast which prevented loss in weight in pigeons fed a B deficient diet supplemented with vitamins B_1 and B_2 (heat stable fractions). They designated it vitamin B_5. It differed from vitamin B_3 in that it was more stable to heat and alkalis. Vitamin B_5 permitted only weight maintenance of the pigeon, and the addition of vitamin B_3 was necessary for increase in weight. There appear to be no other investigations relating to this vitamin.

VITAMIN B_6

Since the recent reports of its isolation, vitamin B_6 must now be listed with the known components of the vitamin B complex. It was defined by Gyorgy[42a] as

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[37] Reader, Vera: The Assay of Vitamin B_4, Biochem. J. **24:** 1827-1831 (No. 6) 1930.

[38] O'Brien, J. P.: Methods of Assay of Vitamin B_4, Chem. & Ind. **53:** 452 (May 18) 1934. Harris, L. J.: Vitamins, Ann. Rev. Biochem. **4:** 331-382, 1935. Kinnersley.[36]

[39] (*a*) Kline, O. L.; Elvehjem, C. A., and Hart, E. B.: Further Evidence for the Existence of Vitamin B_4, Biochem. J. **30:** 780-784 (May) 1936. (*b*) Elvehjem, C. A., and Arnold, Aaron: Interrelationship of Vitamins, Nature **137:** 109-110 (Jan. 18) 1936.

[40] Keenan, J. A.; Kline, O. L.; Elvehjem, C. A.; Hart, E. B., and Halpin, J. G.: New Nutritional Factors Required by the Chick, J. Biol. Chem. **103:** 671-685 (Dec.) 1935.

[41] Barnes, R. E.; O'Brien, J. R., and Reader, Vera: Vitamin B_4, Biochem. J. **26:** 2035-2040 (No. 6) 1932.

[42] Carter, C. W.; Kinnersley, H. W., and Peters, R. A.: Maintenance Nutrition in the Adult Pigeon and Its Relation to Torulin (Vitamin B_1): I, Biochem. J. **24:** 1832-1843 (No. 6) 1930; Maintenance Nutrition in the Adult Pigeon, ibid. **24:** 1844-1851 (No. 6) 1930.

[42a] Gyorgy, Paul: Vitamin B_2 and the Pellagra-like Dermatitis in Rats, Nature **133:** 498-499 (March) 1934.

"that part of the vitamin B complex which is responsible for the cure of a specific dermatitis developed by young rats on the vitamin-free diet supplemented with vitamin B_1 and lactoflavin." Goldberger and Lillie[15] described a dermatitis in rats which was produced when they attempted to demonstrate deficiency of the pellagra-preventive factor with these animals. Later investigations indicate quite definitely that this type of dermatitis is not due to a deficiency of the P-P factor but to some other factor, for which the term B_6[43] has been proposed. While that term is used here, the deficiencies described as being due to factor Y of Chick and Copping,[44] the antidermatitis factor or vitamin H of Hogan and Richardson,[45] vitamin H of Booher,[46] factor I of Lepkovsky, Jukes and Krause[47] and vitamin B_6 appear to be essentially the same.

The cutaneous symptoms developing from the use of diets deficient in vitamin B_6 are described as a symmetrical dermatosis affecting first the paws and tips of the ears and nose. These areas become red, swollen and edematous. It is variously described as florid dermatitis[48] specific type of skin lesion,[49] rat dermatitis,[45b] pellagra-like condition[15] and rat acrodynia.[49]

Vitamin B_6 has been concentrated by fractional adsorption from aqueous extracts of yeast, tikitiki, rice

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43. György, Paul: Vitamin B_2 and Pellagra-Like Dermatitis in Rats, Nature **133:** 498-490 (March 31) 1934.

44. Chick, Harriette, and Copping, Alice Mary: The Composite Nature of the Water-Soluble Vitamin B: III. Dietary Factors in Addition to the Antineuritic Vitamin B_1 and the Antidermatitis Vitamin B_2, Biochem. J. **24:** 1764-1779 (No. 6) 1930.

45. (*a*) Hogan, A. G., and Richardson, L. R.: Differentiation of the Antidermatitis Factor, Science **83:** 17-18 (Jan. 3) 1936. (*b*) Richardson, L. R., and Hogan, A. J.: Skin Lesions of the Rat Associated with the Vitamin B Complex, Bull. 241, Missouri Agri. Exper. Sta. Research.

46. Booher, Lela E.: The Concentration and Properties of Vitamin H, J. Biol. Chem. **119:** 223-231 (June) 1937.

47. Lepkovsky, Samuel; Jukes, T. H., and Krause, Myrtise E.: The Multiple Nature of the Third Factor of the Vitamin B Complex, J. Biol. Chem. **115:** 557-566 (Sept.) 1936.

48. Chick, Harriette; Copping, Alice Mary, and Edgar, Constance Elizabeth: The Water-Soluble B Vitamins: IV. The Components of Vitamin B_2, Biochem. J. **29:** 722-734 (March) 1935. Copping, Alice Mary: The Water-Soluble B-Vitamins: V. Note on the Two Types of Skin Lesion Occurring in Vitamin B_1 Deficiency in the Rat in Relation to Flavin and Vitamin B_6, Respectively; VI. Flavin and Vitamin B_6 in Cereals, Biochem. J. **30:** 845-856 (May) 1936.

49. György, Paul: Investigations on the Vitamin B_2 Complex: I. The Differentiation of Lactoflavin and the "Rat Antipellagra" Factor; II. The Distribution of Lactoflavin and of the "Pellagra-Preventing Factor" (Vitamin B_6) in Natural Products of Animal Origin; III. The Inactivation of Lactoflavin and Vitamin B_6 by Visible Light, Biochem. J. **29:** 741-755 (March) 1935.

bran, fish muscle and wheat germ,[50] by treatment with a large quantity of fullers' earth at p_H 2.5 to 5.0 after the removal of vitamin B_1 and flavin. From such concentrates a compound has been obtained in crystalline form and its empirical formula has been reported to be $C_8H_{12}NO_3Cl$.[51] It is also adsorbed on activated carbon, from which it can be eluted with n-butyl alcohol.[46] It is described as an alkali heat-stable dialyzable factor absorbed on fullers' earth in acid solutions, destroyed by visible and ultraviolet rays, only slightly soluble in water and precipitated by phosphotungstic acid.[50b]

This dietary factor has been found necessary only in the nutrition of the rat. In limited investigations it has been found to be ineffective in the treatment of human pellagra,[51a] nutritional dermatosis in chicks,[47] and blacktongue in dogs.[52] Whether a single or several compounds are responsible for this type of deficiency is not apparent. The Goldberger diet for rat pellagra has been shown to be deficient in a number of factors, but principally in riboflavin.[53]

THE FILTRATE FACTOR

The name "Filtrate Factor"[54] was proposed provisionally to refer to a member of the vitamin B complex which had been demonstrated in earlier investigations at Cornell and Wisconsin [54a] to prevent a dermatitis in chicks. The term was chosen to refer to the method of preparation of concentrates containing the factor used by Elvehjem and Koehn [55] but has also been used in referring to the fraction containing the vitamin. Subsequent studies, even as late as 1938 [55a] report that filtrate factor preparations are effective in curing black-tongue in dogs or human pellagra.[56] The studies that have been made with nicotinic acid now make it clear that if the term "Filtrate Factor" is to be retained, it should be used only as originally defined, viz.: a factor which prevents a nutritional dermatitis (or perhaps preferably dermatosis) in chicks.

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[50] (*a*) Halliday, Nellie, and Evans, H. M.: On the Fractionation of the Vitamin B_2 Complex from Various Source Materials, J. Biol. Chem. **118:** 255-267 (March) 1937. (*b*) Birch, T. W., and György, Paul: A Study of the Chemical Nature of Vitamin B_6 and Methods of Its Preparation in a Concentrated State, Biochem. J. **30:** 304-315 (Feb.) 1936. (*c*) Richardson and Hogan.[45b] (*d*) Booher.[46] (*e*) Lepkovsky, Jukes and Krause.[47]

[51] (*a*) Lepkovsky, Samuel: Crystalline Factor I, Science **87:** 169-170 (Feb.) 1938; The Isolation of Factor I in Crystalline Form, J. Biol. Chem. **124:** 125-128 (June) 1938. (*b*) Akiyoshi Ichiba, and Kimiyo Michi: Crystalline Vitamin B_6, Sc. Papers Inst. Phys. Chem. Research (Tokyo) **34:** 623-626 (June) 1938. (*c*) Gyorgy, Paul: Crystalline Vitamin B_6, J. Am. Chem. Soc. **60:** 983-984 (April) 1938. (d) Keresztesy, J. C., and Stevens, J. R.: Crystalline Vitamin B_6, Proc. Soc. Exper. Biol. & Med. **38:** 64-65 (Feb.) 1938. (*e*) Kuhn, Richard, and Wendt, Gerhard: Uber Das Antidermatitische Vitamin der Hefe, Ber. Deut. Chem. Geselshaft. **71:** 780-782 (April) 1938.

[51a] Dann, W. J.: The Vitamin G Complex: I. The Nonidentity of Rat Dermatitis Due to Vitamin B_6 Deficiency and the Dermatitis of Human Pellagra, J. Nutrition **11:** 451-462 (May) 1936.

[52] Birch, T. W.; György, Paul, and Harris, L. J.: The Vitamin B Complex: Differentiation of the Antiblacktongue and the P-P Factors from Lactoflavin and Vitamin B_6 (So-Called Rat Pellagra Factor): Parts I-IV, Biochem. J. **29:** 2830-2850 (No. 9) 1935. Fouts, P. J.; Lepkovsky, Samuel; Helmer, O. M., and Jukes, T. H.: Effects of Deficiencies of Rat and Chick Antidermatitis Factor on Puppies on a Synthetic Diet, Proc. Am. Soc. Biol. Chem. J. Biol. Chem. **119:** xxxiv (June) 1937.

[53] (*a*) Elvehjem, C. A., and Koehn, C. J., Jr.: Studies of Vitamin B_2 (G): The Nonidentity of Vitamin B_2 and Flavins, J. Biol. Chem. **108:** 709-728 (March) 1935. (*b*) Richardson and Hogan.[45b] (*c*) Lepkovsky, Jukes and Krause.[47] (*d*) György.[49]

[54] Lepkovsky, Samuel, and Jukes, T. H.: The Effect of Some Reagents on the Filtrate Factor (A Water-Soluble Vitamin Belonging to the Vitamin B Complex and Preventing a Dietary Dermatitis in Chicks), J. Biol. Chem. **114:** 109-121 (May) 1936.

Norris and Ringrose [56a] described a nutritional dermatosis in chicks which was characterized by eruptions about the eyes, mouth and feet. The symptoms could be cured by the addition of autoclaved yeast to the diet. This observation led to further studies on the factor involved.[55]

In attempts to fractionate the whole vitamin B complex, it has been found that if B_1 and riboflavin are first removed, further fractionation can be accomplished by adsorption with fuller's earth. The fraction remaining after such adsorption which is free from B_6 contains the filtrate factor. At this writing the indications are that the factor may soon be identified.

FACTOR W

Recently Elvehjem, Koehn and Oleson [57] have presented evidence of the existence in liver of a thermolabile substance necessary for the growth of the rat, which they believe to be different from any members of the B complex previously described. The name factor W has been proposed for this substance. Further studies by Frost and Elvehjem [58] have shown that the addition of adenine nucleotides and nicotinic acid amide produced growth responses in rats fed on a ration deficient in factor W.

[54a] Kline, O. L.; Keenan, J. A.; Elvehjem, C. A., and Hart, E. B.: The Use of the Chick in Vitamin B_1 and B_2 Studies, J. Biol. Chem. **109:** 295-307 (Dec.) 1932.

[55] Koehn, C. J., Jr., and Elvehjem, C. A.: Further Studies on the Concentration of the Antipellagra Factor, J. Biol. Chem. **118:** 693-699 (May) 1937. Keenan, Kline, Elvehjem, Hart and Halpin.[40] Lepkovsky, Jukes and Krause.[47] Elvehjem and Koehn.[53]
Krause.[47] Elvehjem and Koehn.[53]

[55a] Yudkin, S.; Hawksley, J. C., and Drummond, J. C.: A Case of Pellagra Successfully Treated with a Filtrate Factor Obtained from Liver, Lancet **1:** 253-255 (Jan.) 1938.

[56] Koehn, Elvehjem, et al.[55] Fouts, P. J.; Lepkovsky, Samuel; Helmer, O. H., and Jukes, T. H.: Successful Treatment of Human Pellagra with the "Filtrate Factor," Proc. Soc. Exper. Biol. & Med. **35:** 245 (Nov.) 1937. Goldberger, Joseph, and Wheeler, G. A.: Experimental Blacktongue of Dogs and its Relation to Pellagra, Pub. Health Rep. **43:** 172-217 (Jan. 27) 1928.

[56a] Norris, L. C., and Ringrose, A. T.: The Occurrence of a Pellagrous-like Syndrome in Chicks, Science **71:** 643 (June 20) 1930.

[57] Elvehjem, C. A.; Koehn, C. J., Jr., and Oleson, J. J.: A New Essential Dietary Factor, J. Biol. Chem. **115:** 707-719 (Oct.) 1936.

COMMENT

Demonstration of the function of nicotinic acid, isolation of vitamin B_6, and clarification of the position of the filtrate factor all during the past fifteen months have brought kaleidoscopic changes in the cataloging of the components of the vitamin B complex. Four members: Vitamin B_1, riboflavin, nicotinic acid, and vitamin B_6, can now be dealt with as chemical compounds. Only two members of the vitamin B complex, vitamin B_1 and the P-P factor, have been unequivocally linked with deficiency diseases in man. The demonstrated function of riboflavin in oxidation reduction systems, its wide distribution in living cells, its demonstrated relation to growth of rats and chicks and the development of cataract in rats [59] indicate that it is probably an important nutritional essential for man. Clinical investigations have led to the conclusion that riboflavin is ineffective in the treatment of human pellagra [60] and animal experimentation leads to the conclusion that lack of this substance is not responsible for the development of blacktongue in dogs [61] or nutritional dermatosis in rats or chicks.[53a]

There is at present no conclusive evidence that blacktongue in dogs as produced on Goldberger and Wheeler's diet is not the analogue of pellagra in man. On the contrary these diseases have been shown to be equally responsive to treatment with nicotinic acid. The cures of these diseases by the administration of filtrate factor preparations may be explained by the fact that the investigators did not demonstrate that their preparations were free from nicotinic acid. Even though patients with pellagra show a dramatic response to treatment with nicotinic acid, it has not been demonstrated that pellagrins do not suffer from other important deficiencies.

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[58] Frost, D. V., and Elvehjem, C. A.: Further Studies on Factor W, J. Biol. Chem. **121:** 255-273 (Oct.) 1937.

[59] Day, P. L.; Darby, W. J., and Langston, W. C.: The Identity of Flavin with the Cataract-Preventive Factor, J. Nutrition **13:** 389-399 (April) 1937.

[60] Harris, L. J.: Flavin and the Pellagra-Preventing Factor as a Separate Constituent of a Complex B_2, Biochem. J. **29:** 776-781 (March) 1935. Dann.[51]

[61] Sebrell, W. H.; Hunt, D. J., and Onstott, R. H.: Lactoflavin in the Treatment of Canine Blacktongue, Pub. Health Rep. **52:** 235-239 (Feb. 26) 1937.

The relation of vitamins B_3 and B_5 to other members of the B complex is not clear. If the nutritional requirements of the pigeon are similar to those of another avian, the chicken, a reassessment of the importance of these factors will be necessary to catalogue them properly with other factors. Since the original work on vitamin B_4 seems to have been disproved and attempts to confirm more recent work on this factor have not been reported and factor W has not been associated with any deficiency syndrome, the importance of these factors is also rather obscure.

Glossary

vitamin B. 1. The antiberiberi vitamin—American nomenclature. 2. The vitamin B complex—British nomenclature.

vitamin B_1. The antiberiberi vitamin—British terminology, almost universally used at present.

aneurin. Same as vitamin B_1.

thiamin. Same as vitamin B_1.

riboflavin. A compound identified as 6,7 dimethyl-9 (d 1' ribityl) isoalloxazine and essential for growth in chicks and rats and for the prevention of cataracts in rats.

P-P factor. The nutritional factor effective in the prevention of human pellagra (Goldberger).

nicotinic acid. Probably synonymous with P-P factor.

vitamin B_2. 1. That fraction of the B complex other than B_1—British. 2. Synonymous with flavin, riboflavin and vitamin G. 3. The pellagra-preventive factor or factors believed to be identical with it which prevent blacktongue in dogs and a nutritional dermatosis in chicks. 4. That part of the B complex consisting of riboflavin and B_6.

vitamin G. Same as vitamin B_2 but sometimes used to refer to riboflavin plus vitamin B_6.

vitamin B_3. 1. A growth factor for the pigeon. 2. A factor capable of curing specific paralytic symptoms in the rat and later called B_4.

vitamin B_4. A factor associated with specific paralytic symptoms in rats and chicks.

vitamin B_5. A weight maintenance factor for the pigeon.

vitamin B_6. 1. An antidermatitis factor for rats (György). 2. Vitamin B_6 has properties common to but is not necessarily identical with vitamin H of Kuhn and associates, of Sherman and Booher and of Hogan, Factor Y of Chick and Copping, Factor I of Lepkovsky and Jukes and antidermatitis factor of Hogan.

factor Y. See *vitamin B_6.*

vitamin H. See *vitamin B_6.*

vitamin F. Synonymous with B_1 (Sherman); no longer used to designate any factor of the vitamin B complex.

factor W. A growth essential for the rat.

factor I. That part of the B complex other than B_1 and riboflavin which is absorbed by fullers' earth under specified conditions. Contains B_6.

filtrate-factor. A factor effective in the prevention of nutritional dermatosis in chicks.

CHAPTER VII

THE CHEMISTRY OF THIAMIN (VITAMIN B_1)

ROBERT R. WILLIAMS, M.S., D.Sc.

NEW YORK

A few years ago there appeared to be a hopeless conflict among the more or less empirical observations of the host of workers in the field of vitamin B. Fortunately the converging events since 1935 have established the chemical structure and certain properties of vitamin B_1 in a firm fashion. In order to emphasize that this factor is a definite chemical entity, distinct from all other substances, it may be well to use a distinctive name. For years the term antineuritic or antiberiberi vitamin had been used to designate this substance, but such names contain implications contrary to fact or beyond our knowledge; for example, that the relationship between neuritis and beriberi and this vitamin is mutually exclusive. In addition the name "vitamin" is subject to the objection that it brings under a common designation a group of substances which are chemically wholly unrelated to the best of our knowledge.

Prof. B. C. P. Jansen of Amsterdam, who, with W. F. Donath, first isolated the substance from natural sources in 1926,[1] suggested the name aneurin[2] for vitamin B_1. As a well deserved mark of respect for Professor Jansen, this name has come into extensive use in England and continental Europe. However, the name has been objected to on the ground of therapeutic implication and also because of its resemblance to the term aneurysm, which has long been used for a totally unrelated phenomenon. The latter objection seems to me more cogent. As to the former, a therapeutic implication, if based on genuine scientific information, has at least the merit of a memory aid. Many physicians must become confused by the arbitrary letters

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[1] Jansen, B. C. P., and Donath, W. F.: Mededeel. Dienst. Volksgezondheid Nederland. Indie, 1926, part 1, p. 186.

[2] Jansen, B. C. P.: Nature **135**: 267, 1935.

A, B, C and so on for the vitamins. In an endeavor to promote the adoption of a universally acceptable term, based on the chemistry of the substance, I have proposed "thiamin" (chloride, bromide, sulfate and so on) pending action of the Conference on Vitamin Standardization.

THE STRUCTURE OF THIAMIN

The greater part of the labor which has been expended on the chemistry of this substance had to do with its isolation. In addition to the difficulties due to its occurrence in the proportion of only 20 to 30 parts per million in the richest food sources, to its sensitivity to reagents and heat and to its nonselective solubilities, there were complications of confusion with physiologically similar substances, of shortcomings of bio-assays and of lack of the guidance of any general chemical theory. For these reasons the first isolation cost seventeen years of work in many lands, and adequate yields were not obtained until twenty-four years after the first serious attempts at chemical isolation were begun.

Once a few grams of the vitamin became available,[3] its structure was fully established in the relatively short space of three years and its synthesis followed almost immediately. As a participant throughout the several stages of the work I cannot in retrospect blame myself or my colleagues for the long delay. This was inherent in the nature of the problem.

Our knowledge of the structure of thiamin follows largely from the fact that it is quantitatively split by neutral sulfite solution at room temperature according to the equation [4]

$$(1)\quad C_{12}H_{18}N_4SOCl_2 + Na_2SO_3 \rightarrow \underset{\text{I}}{C_6H_9N_3SO_3} + \underset{\text{II}}{C_6H_9NSO} + 2NaCl$$

Product I yields ammonia and a second derivative, III, on strenuous hydrolysis with acids: [5]

$$(2)\quad C_6H_9N_3SO_3 + H_2O \rightarrow \underset{\text{III}}{C_6H_8N_2SO_4} + NH_3$$

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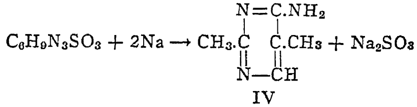
[3] Williams, R. R.; Waterman, R. E., and Keresztesy, J. C.: Larger Yields of Crystalline Antineuritic Vitamin, J. Am. Chem. Soc. **56:** 1187 (May) 1934.

[4] Williams, R. R.: Structure of Vitamin B, J. Am. Chem. Soc. **57:** 229 (Jan.) 1935. Williams, R. R.; Waterman, R. E.; Keresztesy, J. C., and Buchman, E. R.: Studies of Crystalline Vitamin B_1: III. Cleavage of Vitamin with Sulfite, ibid. **57:** 536 (March) 1935.

[5] Williams, R. R.; Buchman, E. R., and Ruehle, A. E.: Studies of Crystalline Vitamin B_1: VIII. Sulfite Cleavage. II. Chemistry of the Acidic Product, J. Am. Chem. Soc. **57:** 1093 (June) 1935.

On treatment with water at 200 C., I (or III) yields sulfuric acid; with alkali, sulfurous acid, thus proving the presence of a sulfonic group in both. Both I and III exhibit absorption in the ultraviolet suggestive of a pyrimidine nucleus. This suggestion was later confirmed by converting I into 2, 5-dimethyl-6-aminopyrimidine, IV, by the action of metallic sodium in liquid ammonia:[6]

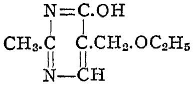
(3) 

$$\mathrm{C_6H_9N_3SO_3} + 2\mathrm{Na} \rightarrow \begin{array}{c}\mathrm{N{=}C.NH_2}\\ \mathrm{CH_3.C\quad C.CH_3}\\ \mathrm{N{-}CH}\end{array} + \mathrm{Na_2SO_3}$$

IV

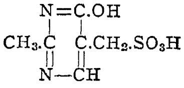
IV was previously unknown but its synthesis was effected by a method adapted from the literature[7] such as to establish its structure. Comparison with the substance obtained from the vitamin served to establish their identity.

By a similar synthesis we[8] obtained 2-methyl-5-ethoxymethyl-6-oxypyrimidine, V, which on treatment with sodium sulfite yielded 2-methyl-6-oxypyrimidine-5-methyl sulfonic acid, VI (equation 4), which proved identical[6] with III (equation 2), whence one could reconstruct the primary cleavage product, I (equation 1), as having structure VII.

(4) 

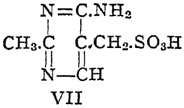
$$\begin{array}{c}\mathrm{N{=}C.OH}\\ \mathrm{CH_3.C\quad C.CH_2.OC_2H_5}\\ \mathrm{N{-}CH}\end{array}$$

V



$$\begin{array}{c}\mathrm{N{=}C.OH}\\ \mathrm{CH_3.C\quad C.CH_2.SO_3H}\\ \mathrm{N{-}CH}\end{array}$$

VI



$$\begin{array}{c}\mathrm{N{=}C.NH_2}\\ \mathrm{CH_3.C\quad C.CH_2.SO_3H}\\ \mathrm{N{-}CH}\end{array}$$

VII

The structure of one of the two main fragments of the vitamin was thus established.

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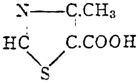
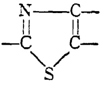
[6] Williams, R. R.: Structure of Vitamin B_1, J. Am. Chem. Soc. **58**: 1063 (June) 1936. Cline, J. K.; Williams, R. R.; Ruehle, A. E., and Waterman, R. E.: Studies of Crystalline Vitamin B_1: XVI. Identification of the Pyrimidine Portion, ibid. **59**: 530 (March) 1937.

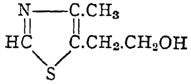
[7] Williams, R. R.; Ruehle, A. E., and Finkelstein, Jacob: Studies of Crystalline Vitamin B_1: XV. C-Methylated 6-Amino- and 6-Oxypyrimidines, J. Am. Chem. Soc. **59**: 526 (March) 1937.

[8] Cline, J. K.; Williams, R. R., and Finkelstein, Jacob: Studies of Crystalline Vitamin B_1: XVII. Synthesis of Vitamin B_1, J. Am. Chem. Soc. **59**: 1052 (June) 1937.

The other fragment, II (equation 1), was more readily identified. On oxidation with nitric acid[9] it yielded 4-methyl-thiazole-5-carboxy acid, VIII, a substance which was first prepared synthetically in 1890.[10] The same substance had also been obtained by Windaus, Tschesche and Grewe[11] by oxidizing thiamin itself with nitric acid. Windaus, however, failed to recognize the thiazole nucleus, IX, which it contained. Recognition of this nucleus[12] by the peculiar stability of its sulfur atom was due to Dr. H. T. Clarke, who kindly collaborated with me at this stage of the study. The thiazole nucleus had never before been found in nature and its occurrence in thiamin is to be regarded as highly significant in a biochemical sense. With knowledge of the presence of this nucleus and with the further information that II (equation 1) contained in a side chain an alcoholic hydroxyl which could be readily replaced by nonionic chlorine[9] without alteration of the nucleus, it was a relatively simple matter to reconstruct the remaining vitamin fragment, II (equation 1), as having the structure X. It is called 4-methyl-5-(β-hydroxy) ethyl thiazole.


VIII


IX


X

In the identification of the cleavage products of thiamin, use was made to an unusual extent of comparisons of ultraviolet absorptions. This was possible because such a large proportion of all the atoms of the thiamin molecule are comprised in the two unsaturated nuclei; viz., pyrimidine and thiazole. Such nuclei have somewhat distinctive absorptions in the ultraviolet. Another physical method, that of potentiometric titration with alkali, was destined to disclose the mode of linkage of the two nuclei to each other.

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[9] Buchman, E. R.; Williams, R. R., and Keresztesy, J. C.: Studies of Crystalline Vitamin B_1: X. Sulfite Cleavage. III. Chemistry of the Basic Product, J. Am. Chem. Soc. **57**: 1849 (Oct.) 1935.

[10] Wöhmann, M.: Ann. d. Chem. **259**: 299, 1890.

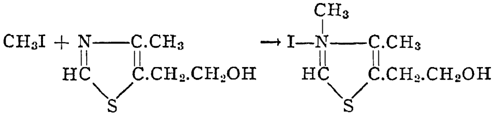
[11] Windaus, A.; Tschesche, R., and Grewe, R.: Ueber das antineuritische Vitamin, Ztschr. f. physiol. Chem. **228**: 27 (Oct. 3) 1934.

[12] Clarke, H. T., and Gurin, S.: Studies of Crystalline Vitamin B_1: XII. The Sulfur-Containing Moiety, J. Am. Chem. Soc. **57**: 1876 (Oct.) 1935.

The point of attachment on the pyrimidine portion was marked by the position of the sulfonic group which was introduced during the sulfite cleavage of thiamin. The point of attachment on the thiazole was less conspicuously marked. It was identified in the following way: Thiamin chloride on being titrated with alkali behaved in a peculiar manner.[13] After the addition of 1 mole of alkali, the alkalinity of the solution no longer rose steadily with further additions. Rather the alkalinity rose only temporarily after each addition and then fell again during the course of a few minutes, indicating an internal rearrangement of the molecule taking place not instantly as ionic reactions do but relatively slowly. Furthermore, this deportment continued until a total of 3 moles of alkali had been added. Only then was there a marked and steady rise in alkalinity. The excessive proportion of alkali required was wholly unexpected at the time, as thiamin salts were known to contain only 2 acid radicals.

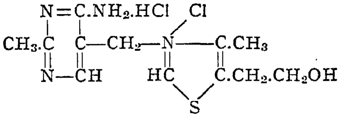
It was found possible to add methyl iodide to X to form XI.[9] Such an addition of methyl iodide is a very general property of tertiary nitrogenous bases and was therefore to be expected in this case. On titration of XI with alkali it

(5)



X XI

showed the same peculiar behavior as thiamin, thus pointing to a fundamental kinship which could be accounted for only by assigning to thiamin the following structure, XII:[6]



XII. Thiamin chloride hydrochloride

The peculiar behavior of these quaternary thiazole salts with alkali is due not to a single rearrangement but to two successive rearrangements of the thiazole portion of the molecule:[14]

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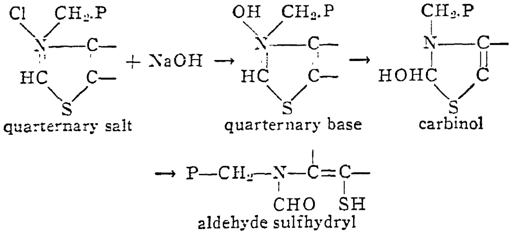
[13] Williams, R. R., and Ruehle, A. E.: Studies of Crystalline Vitamin B1: XI. Presence of Quaternary Nitrogen, J. Am. Chem. Soc. **57**: 1856 (Oct.) 1935.

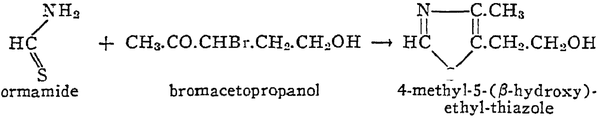


in which P represents in the case of thiamin the pyrimidine nucleus and its substituent groups. The final mole of alkali is required to neutralize the acidic —SH group which is formed when the thiazole ring opens. On addition of acid the series of steps is reversed and the quaternary thiazole, e. g., thiamin, is recovered unchanged. This behavior as well as the sulfite cleavage is an outstanding peculiarity of the unique thiamin molecule. Nature evidently has chosen some aristocratic molecules for the performance of certain of her distinctive biochemical tasks.

SYNTHESIS OF THIAMIN

The first step in the synthesis of thiamin was the reproduction of the thiazole portion. This was first done by my associate Dr. E. R. Buchman[15] by the simple reaction:

(6)



Other syntheses of the same compound also have been achieved.[12]

The synthesis of the pyrimidine portion was accomplished by methods similar to those employed for the production of pyrimidines IV, V and VI, previously discussed in connection with the establishment of structure. The general method is as follows:[16]

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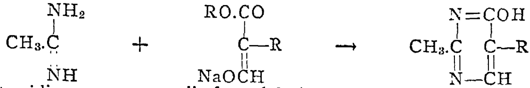
[14] Mills, W. H.; Clark, L. M., and Aeschlimann, J. A.: J. Chem. Soc. **123**: 2353, 1923.

[15] Buchman, E. R.: Studies of Crystalline Vitamin B_1: XIV. Sulfite Cleavage. IV. The Thiazole Half, J. Am. Chem. Soc. **58** 1803 (Sept.) 1936.

(7) 

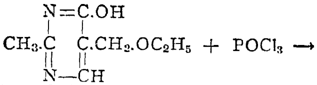
$$\begin{array}{c}NH_2\\ |\\ CH_3.C\\ \vdots\\ NH\end{array} + \begin{array}{c}RO.CO\\ |\\ C{-}R\\ \|\\ NaOCH\end{array} \longrightarrow \begin{array}{c}N{=}COH\\ |\quad\ |\\ CH_3.C\quad C{-}R\\ \|\quad\ \|\\ N{-}CH\end{array}$$

acetamidine

sodio-formyl derivative of substituted ethyl acetate

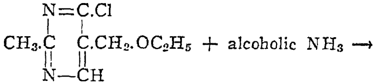
6-oxypyrimidine derivative

If in the foregoing sodioformyl derivative R represents CH_3, the pyrimidine obtained is 2, 5 dimethyl-6-oxypyrimidine; if R represents $CH_2OC_2H_5$ the resulting pyrimidine is V (equation 4). For the synthesis of thiamin itself, product V is converted into a brom amino derivative, thus:



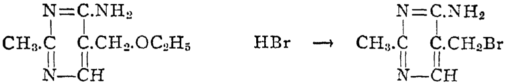
$$\begin{array}{c}N{=}C.OH\\ |\quad\ |\\ CH_3.C\quad C.CH_2.OC_2H_5\\ \|\quad\ \|\\ N{-}CH\end{array} + POCl_3 \longrightarrow$$

V. (2-methyl-5-ethoxy-methyl-6-oxypyrimidine)



$$\begin{array}{c}N{=}C.Cl\\ |\quad\ |\\ CH_3.C\quad C.CH_2.OC_2H_5\\ \|\quad\ \|\\ N{-}CH\end{array} + \text{alcoholic } NH_3 \longrightarrow$$

corresponding chlor-pyrimidine



$$\begin{array}{c}N{=}C.NH_2\\ |\quad\ |\\ CH_3.C\quad C.CH_2.OC_2H_5\\ \|\quad\ \|\\ N{-}CH\end{array} \quad HBr \longrightarrow \begin{array}{c}N{=}C.NH_2\\ |\quad\ |\\ CH_3.C\quad C.CH_2Br\\ \|\quad\ \|\\ N{-}CH\end{array}$$

corresponding amino pyrimidine

XIII. (2-methyl-5-brommethyl 6-aminopyrimidine)

The last of this series of steps is somewhat analogous to that of equation 4. The purpose of putting the Br in the side chain in position 5 instead of the sulfonic group is that the brom compound XIII is very reactive, while the corresponding sulfonic compound VII is extremely unreactive. My associate Dr. J. K. Cline was in large measure responsible for the success of these pyrimidine syntheses and the final condensation.

The final step in the synthesis of thiamin is to heat the hydrobromide of the brompyrimidine, XIII, with the thiazole, X. This results in combination of the two molecules to form thiamin bromide hydrobromide quite analogous to the reaction shown in equation 5.

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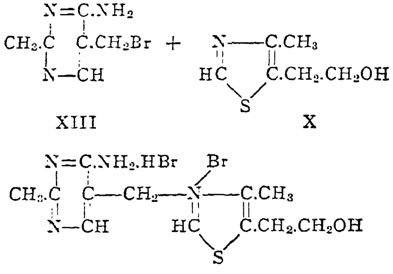
[16] Williams, R. R., and Cline, J. K.: Synthesis of Vitamin B_1, J. Am. Chem. Soc. **58**: 1504 (Aug.) 1936. Cline, Williams and Finkelstein.[8]

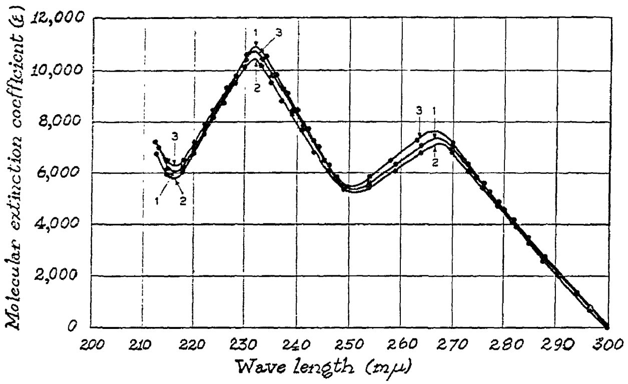
(8)



XIII X

XIV. Thiamin bromide hydrobromide

The bromide hydrobromide, XIV, is readily converted into the chloride hydrochloride, XII, simply by shaking a solution of the former with silver chloride.



A comparison of the ultraviolet absorptions of the natural and synthetic products: curve 1, natural vitamin (hydrochloride); curve 2, synthetic vitamin (hydrochloride); curve 3, synthetic vitamin (hydrobromide).

The resulting substance is chemically identical[17] with the thiamin chloride previously obtained from nature. The correspondence of the ultraviolet absorption spectrums of the two synthetic salts with that from nature is shown in the accompanying chart. Its physiologic action is also identical.[18]

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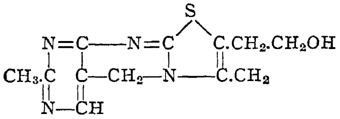
[17] Williams, R. R., and Cline, J. K.: Synthetic Vitamin B_1, J. Am. Chem. Soc. **59**: 216 (Jan.) 1937.

[18] Eckler, C. R., and Chen, K. K.: Action of Synthetic Vitamin B_1, Proc. Soc. Exper. Biol. & Med. **35**: 458 (May) 1937.

Another synthesis of thiamin is given by Todd and Bergel,[19] which involves formation of the thiazole ring upon the preformed appropriate pyrimidyl 5 thioacetamide. Priority has since been claimed on both types of synthesis by Andersag and Westphal whose work was announced but not described by Grewe[20] in 1936. All syntheses agree as to the structure. This structure of thiamin is also confirmed by establishment of the structure of thiochrome, a fluorescent coloring matter first isolated from yeast,[21] later obtained by the oxidation of thiamin[22] and finally synthesized directly by Todd, Bergel and Jacob.[23] Thiochrome has the structure XV. It is possible that thiochrome plays a role as an intermediate in the functioning of thiamin in tissues. I tentatively prefer the view that thiochrome is merely a natural degradation product of thiamin which has already passed the useful stage. It is inactive.



XV. Thiochrome

BIOCHEMISTRY OF THIAMIN

The reactions of thiamin are important particularly for three reasons: (1) They may result in loss of the natural supply by improper methods of distributing and preparing foods; (2) they may serve as a basis for the devising of adequate chemical methods of assaying foods, concentrates and body secretions; (3) they are of interest for the interpretation of the mode of functioning of the substance in living tissues.

Under the first head the most important consideration is the lability of thiamin in neutral mediums. This lability is well exemplified by the ease of cleavage by sulfite at p_H 5 to 6. This cleavage is not peculiar to solutions containing sulfite. Barium nitrite or sodium acetate appears, under certain conditions, to effect splitting in a similar sense and it seems probable that in neutral foods free thiamin is very subject to corresponding splitting under influences which are as yet imperfectly understood. At high acidity this type of cleavage of thiamin does not occur. Probably other constituents of tissues and fluids often participate in destructive reactions when the medium is neutral. Since thiamin is never present in more than a few parts per million, other constituents are in general present in overwhelming proportions.

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[19] Todd, A. R., and Bergel, F.: J. Chem. Soc., March 1937, p. 364.

[20] Grewe, Rudolf: Ueber das antineuritische Vitamin, Ztschr. f. physiol. Chem. **242**: 89 (Aug. 3) 1936. Andersag, H., and Westphal, K.: Ueber die Synthese des antineuritischen Vitamins, Ber. Deutsche chem. Gesellsch. **70**: 2035, 1937.

[21] Kuhn, R.; Rudy, H., and Wagner-Jauregg, T.: Ber. Deutsche. chem. Gesellsch. **66**: 1950, 1933.

[22] Barger, G.; Bargel, F., and Todd, A. R.: Nature **136**: 259, 1935; Ber. Deutsche chem. Gesellsch. **68**: 2257, 1935. Kuhn, R., and Vetter, H. Ber. Deutsche chem. Gesellsch. **68**: 2384, 1935.

[23] Todd, A. R.; Bergel, F., and Jacob, A.: J. Chem. Soc., 1936, p. 1555.

In spite of this fact, loss of B_1 activity in cooking of foods is sometimes negligibly small. Quite possibly the stability of thiamin in foods is to a great extent due to its existence in combined form, e. g., the pyrophosphoric ester, which will be discussed later. Loss by leaching during cooking is probably more serious than by heat destruction, though the latter is also substantial if cooking is prolonged for hours or is carried out at temperatures above 100 C., especially if the reaction of the foodstuff is not markedly acid. We have yet much to learn on this subject but cannot progress greatly till better methods of assay are available.

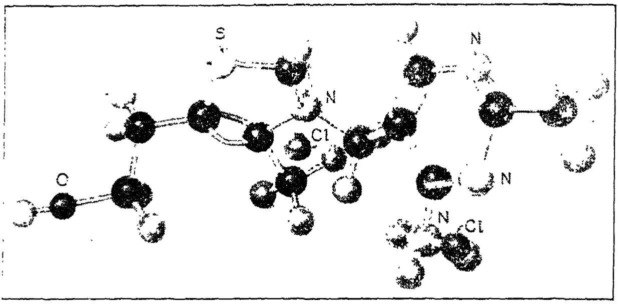


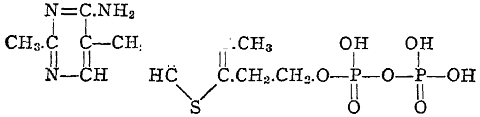
Fig. 1.—Model of thiamin chloride molecule. Nitrogen, oxygen, sulfur, and chlorine atoms are lettered; the others are carbon (black) and hydrogen (white).

The effect of heat on thiamin is serious only in neutral or alkaline solutions. Probably heat merely aggravates the splitting tendency. Solutions of pure thiamin chloride hydrochloride may be sterilized without loss by heating to 120 C. for a half hour or more.[24] This stability is due to the acidity of this salt, which gives solutions of approximately p_H 3.5.

The oxidation of thiamin to thiochrome has already been mentioned. The importance or extent of .this reaction in the foods or in the body is quite unknown. Reduction of thiamin has also been studied by Lipmann [25] and is rather suggestive of a possible mode of functioning of thiamin in living things. Reduction of thiamin with platinum black results in the addition of two hydrogen atoms; reduction with hydrosulfite adds one hydrogen and produces a mole of acid. A similar contrast in the behavior of these two reducing agents is found in their action on a dehydrogenating coferment isolated from red blood cells by Warburg and his co-workers.[26] This suggests a possible dehydrogenase function of thiamin.

BIOCHEMICAL LESION IN THIAMIN DEFICIENCY

A more tangible and better defined relationship of thiamin to an enzyme is found in the work of Löhmann and Schuster,[27] who isolated from yeast a crystalline derivative of thiamin having the structure:



It is the pyrophosphoric ester of thiamin. This substance is split also by sulfite to form the same products which thiamin yields, except that the thiazole is obtained in pyrophosphorylated form rather than free. The ester is approximately as antineuritic as thiamin. It also functions as a cocarboxylase with a protein or proteins present in washed yeast cells and in such an enzyme system strongly promotes the evolution of carbon dioxide from a pyruvate-phosphate medium in

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24. Molitor, H., and Sampson, W. L.: E. Merck, Jahresbericht, 1936.

25. Lipmann, Fritz: Hydrogenation of Vitamin B_1, Nature **138**: 1097 (Dec. 26) 1936.

26. Warburg, Otto; Christian, Walter, and Griese, Alfred: Wasserstoffübertragendes Co-Ferment, seine Zusammensetzung und Wirkungsweise, Biochem. Ztschr. **282**:157 (Nov. 21) 1935. Karrer, Paul, and Warburg, Otto: Jodmethylat des Nicotinsäureamids, ibid. **285**: *297 (May 12) 1936.*

27. Löhmann, K., and Schuster, P.: Untersuchungen über die Cocarboxylase, Biochem. Zeit. **294**: *188, 1937.*

which the cells are suspended, a property which is lacking in free thiamin. The cocarboxylase is thought to promote the reaction:

$$\underset{\text{pyruvic acid}}{CH_3.CO.COOH} \rightarrow \underset{\text{acetaldehyde}}{CH_3.CHO} + CO_2$$

The cocarboxylase is oxidized with alkaline ferricyanide to a thiochrome-like substance. One phosphoric acid group is readily hydrolysed off, the second with difficulty.

The cocarboxylase also has the property of promoting oxygen uptake in vitro of slices of polyneuritic brain tissue in a pyruvate medium, a property previously shown by Peters to be characteristic of pure thiamin. The work of Peters and his associates on this subject is contained in a large number of papers, but since the ideas of the authors have undergone a number of changes the reader will do well to refer first to the latest papers.[28] Those in *Current Science* and the *Lancet* are summaries. The principal finding is that as polyneuritis develops in pigeons the brain tissue loses its power to respire and pyruvic acid accumulates in it. Administration of thiamin corrects these faults in the animal. It also destroys pyruvic acid and promotes respiration of the tissue in vitro. Pyruvic acid is regarded as a normal intermediary in carbohydrate metabolism, which is not properly disposed of in the polyneuritic state. This abnormality is not wholly confined to the brain tissue but is present to some extent in the kidney and heart muscle.

Recently a paper by Westenbrink and Pollak[29] has brought Peters' older observations into even close relationship with the later ones of Löhmann and Schuster. This paper confirms the tissue respiration work of Peters and his associates and adds the interesting observation that there is a ten minute induction period before oxygen uptake begins after the addition of thiamin to a medium containing polyneuritic brain tissue. Westenbrink and Pollak postulate the necessity of thiamin combining with something else before it begins to function. Had they seen Löhmann and Schuster's work before publishing their results they

(Dec
1936.

[29] Westenbrink, H. G. K., and Pollak, J. J.: Rec. trav. chim. Pays. Bas. **56:** 315, 1937.

doubtless would have been tempted to assume pyrophosphorylation as the necessary intermediate step.

All these results relating thiamin to the disposal of accumulated pyruvic acid in polyneuritic tissues is of tremendous interest in view of the finding by Platt and Lu[30] of considerable quantities of pyruvic acid in the blood of patients with beriberi. Indeed, it seems almost certain that the disposal of pyruvic acid by an enzymic decarboxylation is one of the functions of thiamin. Thiamin in pyrophosphorylated form may well con-

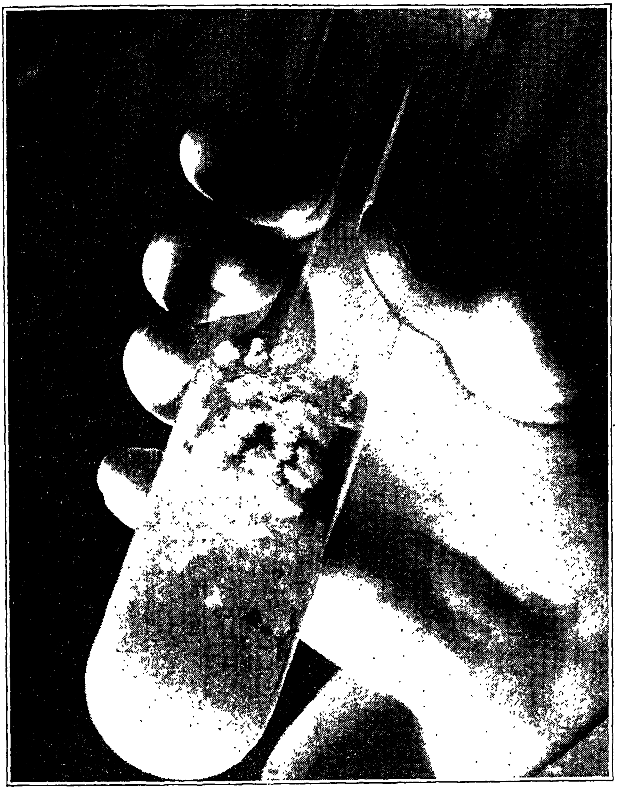

Fig. 2.—Crystals of thiamin chloride.

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[30] Platt, B. S., and Lu, G. D.: Chemical and Clinical Findings in Beriberi, with Special Reference to Vitamin B_1 Deficiency, Quart. J. Med. **5**: 355 (July) 1936.

stitute the prosthetic group of this enzyme just as riboflavin, B_2, in phosphorylated form constitutes the prosthetic group of a dehydrogenating enzyme.[31]

However, many points remain to be cleared up before it can be said that this is the sole or even principal function of thiamin. Particularly it is not yet clear precisely how promotion of oxygen uptake is related to decarboxylation. In addition there is an extensive literature which attempts to associate methyl glyoxal (pyruvic aldehyde) rather than pyruvic acid with the beriberic condition. Peters found no methyl glyoxal in polyneuritic brain tissue of pigeons,[32] but it has been reported by others in the urine of B_1 deficient infants,[33] of B_1 deficient rats [34] and in liver extracts of B_1 deficient animals.[35] Moreover, there is an extended series of voluminous articles of Japanese origin chiefly in the Tohoku *Journal of Experimental Medicine* during the past fifteen years which greatly emphasizes the occurrence in the milk of mothers with beriberi of a methyl glyoxal-like substance and also a deficiency of a normal peroxidase in the milk and blood leukocytes. The Japanese authors emphasize the toxic character of this milk. Mice on a polished rice diet die in from one to five days if injected daily with milk from mothers with beriberi or rabbits with polyneuritis, while normal milk has no effect.[36] In women, one breast may secrete toxic milk, the other nontoxic.[37] There are from seventy-five to eighty papers along these lines in this one journal during the past fifteen years. They deserve careful study but have been generally neglected. One of the most impressive papers is by Chiba,[38] as it gives detailed data on many scores of human beriberi cases.

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[31] Tauber, Henry: Enzyme Chemistry, New York, John Wiley & Sons, Inc., 1937.

[32] Peters, R. A.: Current Science **5:** *207, 1936.*

[33] Geiger, A., and Rosenberg, A.: Methylglyoxal im Harne und in der Cerebrospinalflüssigkeit bei Ernährungsstorungen der Säuglinge mit toxischen Symptomen und bei der experimentellen B_1-Avitaminose bei Hunden und Ratten, Klin. Wchnschr. **12:** 1258 (Aug. 12) 1933.

[34] Lehmann, Jörgen: Ist das Vorkommen von Methylglyoxal im Harn Spezifisch für B_1-Avitaminose? Skandinav. Arch. f. Physiol. **71:** 157 (Feb.) 1935.

[35] Vogt-Møller, J.: Ist Avitaminosis B_1, eine Intoxikation mit Methylglyoxal? Biochem. Ztschr. **233:** 248 (April 22) 1931.

[36] Asakura, Kyumatsu, and Ohsako, Hujio: Arakawa's Reaction and Toxicity of Human Milk. Tohoku J. Exper. Med. **20:** 429 (March) 1933.

[37] Asakura, Kyumatsu: Relation Between Arakawa's Reaction and Suitability of Human Milk, Tohoku J. Exper. Med. **19:** 275 (Aug.) 1932.

[38] Chiba, Marié: Prolongation of Short-Timed Peroxidase Reaction of Leukocytes as a Sign of Avitaminosis B and Effect of Vitamin B on It: A Clinical Experiment, Tohoku J. Exper. Med. **19:** 486 (Sept.) 1932.

The function of thiamin is probably not merely to get rid of pyruvic acid but to utilize it in some metabolic process. Thiamin has been shown to play a fundamental role in the physiologic economy of plants. It promotes alcoholic fermentation by yeast;[39] it is essential to growth of excised embryos of seeds influencing root production profoundly.[40] According to the results of W. J. Robbins, Mary H. Bartley,

Fig. 3.—Synthetic vitamin B_1 is now available in quantity. Train loads of rice polish would be needed to produce an equivalent amount by isolation.

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[39] Schultz, Alfred; Atkin, Lawrence, and Frey, C. N.: A Fermentation Test for Vitamin B_1, J. Am. Chem. Soc. **59:** 948 (May) 1937.

[40] Kögl, Fritz, and Haagen-Smit, A. J.: Biotin und Aneurin als Phytohormone. Ztschr. f. physiol. Chem. **243:** 209 (Oct. 14) 1936. Bonner, James: Vitamin B_1 a Growth Factor for Higher Plants, Science **85:** 183 (Feb. 12) 1937. Robbins, W. J., and Bartley, M. H.: Vitamin B_1 and the Growth of Excised Tomato Roots, Science **85:** 246 (March 5) 1937.

A. G. Hogan and others,[41] plants and, to some extent, animals have a capacity for synthesizing thiamin if supplied with the intermediates used in its artificial synthesis. Thiamin functions in the economy of a wide variety of micro-organisms, in the lower and higher plants, and in insects as well as in all higher animals. One can scarcely regard it otherwise than as one of nature's earlier and more fundamental inventions in the process of evolving life. It is difficult to believe that its action in the higher forms of life is restricted to specialized tissues or to a narrow function.

METHODS OF ASSAY AND DISTRIBUTION IN FOODS

The existing methods of assay are wholly biologic in nature and are not entirely satisfactory. The method most widely used for testing foods is the rat growth method. It gives fairly consistent results but cannot be highly specific as it involves the unwarranted assumption that there are no thermolabile vitamins in yeast or in foods generally other than thiamin. The rat curative method [42] is more specific but it has not been developed or even tried extensively for food testing purposes. Also the international standard of reference is an acid clay adsorbate which, because of the firm combination of thiamin with the clay, may not yield its thiamin fully in animal assays,[43] the availability varying according to the condition of the animals. This has led to endless confusion.

It will be no easy matter to devise a satisfactory method of chemical assay for thiamin in foods. It occurs in the proportions of only from 0.1 to 4 parts per million, roughly a thousand times less abundant than vitamin C. It possesses no known physical property which is adapted to delicate testing. Jansen [44] has proposed to convert it to thiochrome, which can be estimated optically; but a quantitative conversion has not yet been fully achieved. Another lead is that of McCollum and Prebluda,[45] who couple it with diazotized para-amino-acetophenone to form a red dye which may be estimated colorimetrically. An efficient method of extracting the vitamin from foods will be necessary for the application of such assay methods.

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[41] Robbins, W. J.; Bartley, Mary A.; Hogan, A. G., and Richardson, L. R.: Pyrimidine and Thiazole Intermediates as Substitutes for Vitamin B_1, Proc. Nat. Acad. Sc. **23**:388 (July) 1937.

[42] Smith, M. I.: Pub. Health Rep. **45**: 116 (Jan. 17) 1930.

[43] Sampson, W. L., and Keresztesy, J. C.: Proc. Soc. Exper. Biol. & Med. **36**: 30 (Feb.) 1937.

[44] Jansen, B. C. P.: Rec. trav. chim. Pays. Bas. **55**: 1046, 1936.

[45] Prebluda, H. P., and McCollum, E. V.: A Chemical Reagent for the Detection and Estimation of Vitamin B_1, Science **84**: 488 (Nov. 27) 1936.

DIAGNOSTIC TESTS

It has been proposed to assay the urine as a diagnostic test of thiamin deficiency in patients, and some evidence of success has been cited.[46] It is likely that many data will be necessary to establish suitable norms for diagnostic purposes, even though it is found that there is prevailingly a fixed ratio between ingested thiamin and that excreted in the urine. It may well be that there is a variable destruction in the body or elimination in the feces.

The therapeutic response to thiamin remains the most trustworthy diagnostic test which we have. It is quite safe, as doses of as high as 90 mg. have evidenced no pharmacologic effect in human subjects.[47] Rats have received as high as 5 mg. daily throughout life for three successive generations without adverse effects. This corresponds on a weight basis to about 1 Gm. daily for the human adult. Doses of from 2 to 10 mg. daily for several weeks are therefore extremely conservative for human treatment. The daily intake in food for human adults is of the order of 1 to 2 mg.; the requirement is proportional to the food intake and particularly to the carbohydrate intake.

The diagnostic value of thiamin therapy is largely lost if crude natural preparations are used both because of the uncertainty of their standardization and of the presence of other vitamins which may be responsible for the observed effects. After demonstrating a B_1 deficiency and rectifying the immediate shortage, the physician should endeavor to direct the diet of the patient toward a more adequate supply of the vitamin. In the long run we should look to the grocery store rather than the drug store for a normal intake.

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[46] Harris, L. J., and Leong, P. C.: Vitamins in Human Nutrition, The Excretion of Vitamin B_1 in Human Urine and Its Dependence on the Dietary Intake, Lancet **1**: 886 (April 18) 1936. Helmer, O. M.: Vitamin B_1 and B_2 Content of Human Urine, Proc. Soc. Exper. Biol. & Med. **32**: 1187 (April) 1935. Roscoe, Margaret H.: The B Vitamins in Human Urine, Biochem. J. **30**: 1053 (June) 1936. Jolliffe, N., and Goodhart, R.: The Urinary Excretion of Vitamin B_1. Its Dependency on Dietary Intake in Normal Subjects, Proc. Am. Physiol. Soc., 1938, p. 111.

[47] Vorhaus, M. G.; Williams, R. R., and Waterman, R. E.: Studies on Crystalline Vitamin B_1: Observations in Diabetes, Am. J. Digest. Dis. & Nutrition **2**: 541 (Nov.) 1935.

CHAPTER VIII

THE PHYSIOLOGY OF VITAMIN B_1

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As the papers of this series are planned to be a continuation of those of an earlier series, no attempt will be made here to review at all completely the voluminous literature bearing on the physiology of vitamin B_1. The historical development of the subject, the various methods which may be followed in assaying for this vitamin, the importance of the vitamin for growth. lactation and other functions, and similar topics concerning which our fundamental knowledge has been established for many years—all of these will be omitted from the present discussion. Instead, the objective will be to survey briefly the more recent contributions to knowledge in this field, to summarize as briefly as possible the present points of view concerning the relation of vitamin B_1 to various bodily functions, to describe the unique function of this dietary factor, if there is such, and, finally, to indicate wherever possible the significance in medicine and practical dietetics of our present knowledge.

DISTRIBUTION OF VITAMIN B_1 IN THE BODY

It is conceivable that knowledge of the relative distribution of vitamin B_1 in the body may assist in our understanding of its possible functions. We are concerned here not with the usefulness of such information in relation to relative food values of the various tissues but rather with its bearing on the function of vitamin B_1.

The earlier literature on this subject emphasized a similarity of distribution of the so-called nucleins and vitamin B_1, and on this observation was based the idea that this dietary essential has some important role in the functioning of every cell rather than in the activity of a special organ or tissue system. More recent studies have enlarged the body of available data without seriously altering the conclusions drawn from them.

Westenbrink[1] performed feeding experiments with the tissues of normal and B_1 deficient rats and found the heart, liver and kidney to have the highest content of vitamin B_1 under normal conditions, the last two organs evidently acting as storehouses for the dietary factor. All of the organs examined except the brain were observed to suffer great diminution in their vitamin B_1 content after the rats had subsisted for five weeks on the B_1 free diet. Essentially similar results were obtained by Graham and Griffith.[2] In this connection it is pertinent to say that in 1923 Nagayo[3] cited the presence of vitamin B_1 in the tissues of patients who had died of beriberi as strong evidence for the view that beriberi is not due essentially to vitamin B_1 deficiency. I do not believe that the validity of the deficiency disease theory requires that the tissues become absolutely devoid of the vitamin before characteristic symptoms and pathologic changes may occur, but merely that the concentration in the various organs, or perhaps in certain special organs, must become less than a critical or threshold value. Furthermore, it is reasonable to believe that the different organs will respond to the condition of low vitamin supply in various ways. Such a point of view appears consonant with all the data now at hand.

The amounts of vitamin found in the various tissues under different dietary regimens support the conclusion that the organism's capacity to store vitamin B_1 is limited. In the pigeon, the rat and the dog, the time required for a shortage of vitamin B_1 to become evident varies from about ten days to three or four weeks, the latter period being characteristic of the largest of these species, the dog. This limited capacity for storage is obviously of great importance in relation to clinical situations as well as in practical dietetics and hygiene.

Students interested in the quantitative aspects of this topic will find the data of Brodie and MacLeod[4] worthy of examination. These investigators reported that in animals subsisting on normal diets the liver is found to contain about ten times as much vitamin B_1 per gram as voluntary muscle; the kidney is about one-half and the brain approximately one-third as rich per gram as voluntary muscle. The heart is almost as rich as the liver. Only traces of the vitamin are found in the blood, spleen and lungs. It was found that within certain limits the vitamin B_1 content of the body is undoubtedly influenced by the amount of vitamin in the diet, a conclusion harmonizing well with the results of many other types of investigations.

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[1] Westenbrink, H. G. K.: Ueber den Gehalt an Vitamin-B_1 der Organe von weissen Ratten bei normaler und Vitamin-B_1-freier Ernährung, Arch. néerl. de physiol. **17**: 560-577, 1932.

[2] Graham, Claire, and Griffith, W. H.: Vitamin B_1 and B_2 in Tissues of Normal and Experimental Rats, Proc. Soc. Exper. Biol. & Med. **29**: 695-697 (Feb.) 1932.

[3] Nagayo, Mataro: Beriberi and Rice Neuritis, J. A. M. A. **81**: 1435-1437 (Oct. 27) 1923.

[4] Brodie, Jessie B., and MacLeod, Florence L.: Quantitative Experiments on the Occurrence of Vitamin B in Organs, J. Nutrition **10**: 179-186 (Aug.) 1935.

The suggestions in the literature (1) that vitamin B_1 functions in some way in the metabolism of one or more of the energy-yielding foodstuffs, probably carbohydrate, and (2) that fat in some way "spares" vitamin B_1 (discussed later in this paper) led Westenbrink[5] to study the disappearance of vitamin B_1 from certain organs of pigeons subsisting on B_1-free, carbohydrate-rich, fat-poor diets as compared with those subsisting on B_1-free, carbohydrate-free, fat-rich combinations of food. In view of his results, Westenbrink considered it unlikely that the appearance of polyneuritis after shorter or longer periods of the feeding of large amounts of carbohydrate or fat can be explained as due simply to the more rapid or slower disappearance, respectively, of vitamin B_1 from the organism. Some other factor seems to be involved.

For some time it has been known that vitamin B_1 is present in the urine and that the amount may vary with that furnished in the diet, a plethora of vitamin in the blood and the entire body evidently being associated with increased elimination of the dietary essential through the kidney. As the vitamin is water soluble, its presence in the urine is easily understood. The fact that the amount thus eliminated can vary with that available within the organism is important to the physiologist and the clinician. On it one may base a search for a vitamin B_1 function test by which to detect persons who do not have their tissue reservoirs "saturated" with the vitamin. This fact is also important because on it rests the possibility that vigorous activity of the kidney, as for example in pronounced diuresis, may mean an increased elimination of vitamin B_1 from the body, to its possible detriment. Tests of this possibility by experiments with dogs[6] have in fact yielded positive results.

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[5] Westenbrink, H. G. K.: Ueber das Verschwinden des Vitamins-B_1 aus einigen Organen von Tauben bei Vitamin-B_1-freier, kohlehydratreicher, fettarmer Nahrung, bezw. bei Vitamin-B_1-freier, kohlehydratfreier, fettreicher Nahrung, nebst einer Vergleichung des Vitamin-B_1-Gehaltes von Organen von Ratten und Tauben, Arch. néerl. de physiol. **19**: 116-121, 1934.

Harris and Leong[7] and others have examined experimentally the possibility of a vitamin B_1 function test based on quantitative examination of the urine secreted under definite conditions. The reader is referred to the original papers for details. Let it suffice to state here that, in the opinion of these investigators, "a daily excretion of less than 12 international units (corresponding with an average concentration of 1 I. U. per 100 cc. raises the presumption that the diet contains less than a normal allowance of vitamin B_1." With the isolation and synthesis of pure vitamin B_1, and therefore its availability, and the perfection of microchemical methods by which to analyze the urine for this factor, it is likely that other vitamin B_1 function tests will be devised which are accurate yet more saving of time in their performance. The application of such methods in the survey of patients in the clinic and in representative groups of the normal population should result in numerous interesting and valuable observations.

VITAMIN B_1 AND THE ALIMENTARY TRACT

Recent additions to the literature bearing on vitamin B_1 and the alimentary tract have not been such as to warrant any serious changes in the view held hitherto, that lack of vitamin B_1 does not produce any specific dysfunction of the organs of the alimentary tract. Sure and Thatcher[8] were able to confirm an earlier report[9] that gastric ulcers appear in a large percentage of white rats subsisting on a B_1-deficient diet and therefore suggested that vitamin B_1 therapy may be indicated in the treatment of gastric ulcers in human beings. The failure to secure ulcers in practically every case, however, constitutes an argument in favor of the view that the ulcers arise secondarily in the course of vitamin B_1 deficiency, probably as part of the response of the stomach to a generalized systemic condition and not because the function of vitamin B_1 is specific in relation to this organ.

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[6] Cowgill, G. R.; Rosenberg, H. A., and Rogoff, J.: Studies in the Physiology of Vitamins: XIV. The Effect of Administration of Large Amounts of Water on the Time Required for the Development of the Anorexia Characteristics of a Deficiency of the Vitamin B Complex, Am. J. Physiol. **95**: 537-541 (Dec.) 1930.

[7] Harris, L. J., and Leong, P. C.: Vitamins in Human Nutrition: The Excretion of Vitamin B_1 in Human Urine and Its Dependence on the Dietary Intake, Lancet **1**: 886 (April 18) 1936. Helmer, O. M.: Vitamin B_1 and B_2 Content of Human Urine, Proc. Soc. Exper. Biol. & Med. **32**: 1187 (April) 1935. Roscoe, Margaret H.: The B Vitamins in Human Urine, Biochem. J. **30**: 1053 (June) 1936.

[8] Sure, Barnett, and Thatcher, H. S.: Avitaminosis: XIV. Production of Gastric Ulcers in the Albino Rat as a Result of Specific Influence of Deficiency of Vitamin B, Arch. Path. **16**: 809-816 (Dec.) 1933.

[9] Dalldorf, Gilbert, and Kellogg, Minerva: Incidence of Gastric Ulcer in Albino Rats Fed Diets Deficient in Vitamin B (B_1), J. Exper. Med. **56**: 391-398 (Sept.) 1932.

Babkin[10] has reported interesting observations suggesting a relation of vitamin B_1 or some other member of the B complex to the nervous mechanism controlling gastric secretion. Dogs and cats, suitably operated on so as to permit experiments of the sham feeding type, were allowed to subsist on a diet deficient in the B complex and then were tested for their gastric responses to sham feeding, subcutaneous injections of histamine and the presence of food and 5 per cent alcohol solution in the intestine. During the state of vitamin deficiency there was a marked diminution in the response of the gastric glands to all of these stimuli. When yeast was administered the responses became normal within a few days. The effective agent in the yeast was not determined. These results are of interest because they suggest a possible explanation of the anorexia so characteristic of vitamin B deficiency. It will be recalled that earlier attempts to explain this anorexia by study of the effects of vitamin B deficiency on the so-called hunger contractions of the empty stomach,[11] while yielding interesting results, nevertheless failed to furnish the desired explanation. The origin of this anorexia still remains an unsolved problem.

Studies have been made of the efficiency with which various enzymes of the alimentary tract can function in the presence of vitamin B deficiency. Sure and his associates[12] reported in vitro experiments indicating that no impairment of tryptic and ereptic digestion of casein is associated with B deficiency. Such a result conforms well with those recorded in the earlier literature. With respect to the activity of pancreatic lipase, however, a pronounced decrease was noted. The significance of these in vitro results is questionable in view of the in vivo observations of Salmon and Goodman[13] that in rats there is no impairment in the digestion of fat as a result of vitamin B_1 deficiency.

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[10] Babkin, B. P.: Nervous Control of Gastric Secretion and Effect of Vitamin Deficiency on Its Production, Canad. M. A. J. **29**: 5-9 (July) 1933.

[11] Cowgill, G. R.; Deuel, H. J., Jr.; Plummer, N., and Messer, F. C.: Studies in the Physiology of Vitamins: IV. Vitamin B in Relation to Gastric Motility, Am. J. Physiol. **77**: 389-401 (July) 1926.

[12] Sure, Barnett; Kik, M. C., and Buchanan, Kathryn S.: Enzymic fficiency in Avitaminosis: I. Influence of Vitamin B Deficiency on ryptic and Ereptic Digestion of Casein, J. Biol. Chem. **108**: 19-26 Jan.) 1935; II. Influence of Vitamin B Deficiency on Efficiency of Panreatic Lipase and Esterase, J. Biol. Chem. **108**: 27-33 (Jan.) 1935.

Reder and Gallup[14] reported that rats lacking both vitamin B_1 and vitamin G exhibit slower digestion and absorption of administered carbohydrate than normal control animals. When vitamin B_1 is added to the diet the rate does not improve; however, the addition of vitamin G to the ration proves effective. These results, apart from their significance in relation to the theme under discussion, emphasize to the investigator in this field the importance of planning his experiments so as to be able to attribute his results more specifically to vitamin B_1 or other well defined members of the B complex.

As anorexia is a common characteristic of vitamin B_1 deficiency and its presence necessarily means some degree of inanition, students of the physiology of vitamin B_1 have learned to appreciate the importance in their experiments of making simultaneous observations on control animals receiving the vitamin but restricted in their intake of the basal diet to the amount ingested voluntarily by the B_1-deficient animals. When such control observations are made, it is astonishing how many of the differences from normal function that have been reported have proved to be nonspecific, appearing in the undernourished control animals as well. This proved again to be the case in the recent work reported by Chatterjee,[15] who studied the motor functions of the intestine in the presence of B_1 deficiency. In both the vitamin-deprived and the starved animals there was a definite decrease in the amplitude, the number and the intensity of the intestinal contractions, as well as in the responses to pilocarpine, atropine, nicotine and barium chloride.

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[13] Salmon, W. D., and Goodman, J. G.: Alleviation of Vitamin B Deficiency in the Rat by Certain Natural Fats and Synthetic Esters, J. Nutrition **13:** 477-500 (May) 1937.

[14] Reder, Ruth, and Gallup, W. D.: The Rate of Digestion and Absorption During Avitaminosis B and G, Proc. Oklahoma Acad. Sc. **15:** 58-61, 1935.

[15] Chatterjee, D. D.: Motor Functions of the Bowel in Avitaminosis B and in Starved Animals, Indian J. M. Research **23:** 191-198 (July) 1935.

Molitor and Sampson[16] have tested the influence of pure vitamin B_1 on intestinal motility, using isolated rabbit's intestine suspended in Ringer's solution as well as taking observations of the intestine in situ. The experiments included study of the contractions under normal conditions and after the movements had been paralyzed with papaverine. The addition of pure vitamin to the Ringer solution, as well as the intravenous injection of vitamin in the in situ experiments, was without any effect on the movements. Evidently, then, this agent, unlike various drugs, has no demonstrable effect on the intestine of the normal organism; its action is exerted up to the point required by the animal but not beyond.

Vitamin B_1 is readily absorbed from both the small and the large intestine, and in all probability people may vary with respect to the efficiency of such absorption. When making vitamin B_1 assays with pigeons by a technic involving the administration of materials by way of the crop, I[17] have frequently found a bird which requires much more vitamin B_1 than other comparable birds of a series. This difference may theoretically be due to less efficient absorption, but it might also be the result of a more ready elimination through the kidney or more rapid consumption in metabolic processes. Some observations[18] have been made of the absorption of various vitamin B_1 products when placed in the large intestine of the dog through a valvular cecostomy. From such experiments it appears that vitamin B_1 is not readily eluted from a fullers' earth adsorbate in the large intestine, and such a product therefore is less effective than others which contain the vitamin already dissolved in a suitable aqueous medium. These observations are of significance to clinicians who may desire to give vitamin B_1 to patients having a fistula in the large intestine. Evidently not all vitamin B_1 products are of equal value for such therapeutic use.

Even when administered by mouth, products of the type of fullers' earth adsorbate, such as the present international vitamin B_1 standard, do not give up all of their adsorbed vitamin when passing through the alimentary tract. Sampson and Keresztesy[19] have been able to show that elution with quinine sulfate solution yields approximately twice as much vitamin as elution by the older methods involving alkaline solutions. In the light of these facts it is reasonable to assume that the variable biologic effects reported by workers using adsorbates are due to different degrees of elution of the vitamin within the intestine.

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[16] Molitor, H., and Sampson, W. L.: Personal communication to the author from the Merck Laboratory of Therapeutic Research, Rahway, N. J.

[17] As an illustration see pigeon 25, table 2, page 37, in Cowgill, G. R.: The Vitamin B Requirement of Man, New Haven, Conn., Yale University Press, 1935.

[18] Cowgill, G. R., and Weinstein, L.: Unpublished observations.

It is theoretically possible that vitamin B_1 may be lost from the body under the conditions of chronic diarrhea. This question has been examined experimentally.[20] It appears that diarrhea merely causes some failure of absorption of an appreciable fraction of the ingested vitamin; it does not cause an excretion of vitamin B_1 from the organism through the intestinal wall into the lumen of the intestine.

The presence of vitamin B_1 in the feces has led many investigators to endeavor to determine its origin. In view of the observations of numerous workers it appears likely that this vitamin is synthesized by certain bacteria and other micro-organisms. Bechdel and his associates[21] reported that the cow is able to synthesize vitamin B_1 in its rumen through the activity of certain bacteria and thus derive the benefits of the synthesized products without the necessity of reingestion of feces, as is the case with the rat and other animals. In the case of the rat it appears from the work of Guerrant and his collaborators[22] that the type of carbohydrate in the ration has some role in this process. Sucrose, dextrose and starch, being usually assimilated quite rapidly, rarely reach the cecum in appreciable amounts, and it was shown that the elaboration of vitamin B_1 takes place in this region of the intestine.

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[19] Sampson, W. L., and Keresztesy, J. C.: A Comparison of Some Methods for the Extraction of Vitamin B_1 from International Standard Acid Clay, Proc. Soc. Exper. Biol. & Med. **36:** 30-32 (Feb.) 1937.

[20] Dann, Margaret, and Cowgill, G. R.: The Influence of Diarrhea on the Vitamin B Requirement, to be published.

[21] Bechdel, S. I.; Honeywell, Hannah E.; Dutcher, R. A., and Knutsen, M. H.: Synthesis of Vitamin B in the Rumen of the Cow, J. Biol. Chem. **80:** 231-238 (Nov.) 1928.

[22] Guerrant, N. B.; Dutcher, R. A., and Tomey, L. F.: The Effect of the Type of Carbohydrate on the Synthesis of the B Vitamins in the Digestive Tract of the Rat, J. Biol. Chem. **110:** 233-243 (June) 1935. Guerrant, N. B.; Dutcher, R. A., and Brown, R. A.: Further Studies Concerning the Formation of the B-Vitamins in the Digestive Tract of the Rat, J. Nutrition **13:** 305-313 (March) 1937.

In his summary of this work Dutcher[23] added: "Dextrin, on the other hand, is digested and assimilated at a slower rate, with the result that this carbohydrate reaches the cecum, where organisms (probably yeasts) have an opportunity to multiply. These organisms synthesize the B_1 and G complex, which is not readily assimilated in the lower bowel but is voided in the feces. Lactose occupies an intermediate position between the soluble sugars and dextrin."

Dutcher mentioned yeasts as possible organisms in this connection, but Shimoda[24] reported Bacillus coli as having been proved in his experiments to be able to synthesize vitamin B_1. In this explanation of the results yielded by the trials with different carbohydrates, it is assumed that synthesized vitamin B_1 is thrown out into the semifluid chyme and thus made available for absorption. In some unpublished experiments Weinstein and I grew pure cultures of organisms supplied by Bechdel and reputed to be capable of synthesizing vitamin B_1. Biologic tests of such material for B_1 content led to the conclusion that any vitamin present in the tested products is contained in the bacterial cells instead of being secreted into the medium by the cells. If one is permitted to generalize from this conclusion, it would seem (1) that any vitamin B_1 made available to the host by bacterial action within the large intestine must arise from dead and decomposed bacteria and (2) that variations in the amount of the vitamin found in the feces probably represent variations in the growth of the B_1-containing organisms.

Practically all the tests made of the feces have involved feeding of the excreta to animals, and in this case the bacterial bodies would be subjected to the action of the digestive juices secreted in the upper parts of the alimentary tract. The work of Guerrant and his associates previously cited shows that in the rat the synthesis of vitamin B_1 by micro-organisms takes place in the cecum, and for the animal to obtain the benefit of this elaboration of vitamin it must ingest the feces. On the other hand, if the vitamin could be synthesized in the small intestine by a process resulting in the presence of the vitamin in the liquid chyme outside

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23. Dutcher, R. A.: Recent Trends in Vitamin Research, J. Home Econ. **28**: 621-630 (Nov.) 1936; see page 629.

24. Shimoda, Y., of the Municipal Hygienic Laboratory, Osaka, Japan: personal communication to the author.

the bodies of the synthetically active cells, the gross phenomena presented by the animals would be essentially those characteristic of refection.[25]

VITAMIN B_1 AND THE HEART

Phenomena referable to the heart have long been known as characteristic of beriberi in human beings. It is natural therefore that physiologists should be interested in the relation of vitamin B_1 to cardiac function. The most striking recent contribution to knowledge of this subject[16] is the fact that pure vitamin B_1 has no specific influence on the normal heart; only in the B_1-deficient organism does administration of the vitamin result in a demonstrable effect.

In rats subsisting on B_1-deficient diets bradycardia eventually appears, and Birch and Harris[26] have made this the basis of a technic for assay of vitamin B_1. In this connection, however, it is pertinent to point out that one cannot generalize with respect to the phenomenon of bradycardia, which undoubtedly occurs in the B_1-deficient rat. Unpublished observations by Hoff, Street and me indicate that slowing of the heart rate is not a characteristic feature of vitamin B_1 deficiency in the dog. It is true that the enlargement of the right side of the heart so characteristic of beriberi in human beings occurs with experimental B_1 deficiency produced in different species of animals, but the physiologic response which this organ shows to lack of the vitamin is not the same in all species. The reader interested in this question will find an excellent summary of the facts bearing on the question of the identity of experimental vitamin B_1 deficiency with beriberi in the paper by Shimazono;[27] this author also discussed the differences in the pictures of B_1 deficiency presented by different species of animals.

Aalsmeer and Wenckebach[28] have studied the heart in cases of beriberi and advanced the suggestion that it is essentially an edematous organ. To describe the condition as hypertrophy is certainly incorrect, because the heart can return to normal size within a remarkably short time after institution of vitamin B_1 therapy. The recent observation made in Dr. A. B. Hastings' laboratory at Harvard that, in the presence of B_1 deficiency, the tissue of the auricle, in contrast to that of the ventricle, shows a marked reduction in oxygen uptake from the normal, constitutes proof that these two parts of the heart respond differently to a shortage of vitamin B_1 and that the auricle is more sensitive in this respect than the ventricle. On this basis I offer the suggestion that, because of this greater sensitivity of the auricle, this part of the heart becomes weaker, loses tone and as a result suffers greater mechanical distention in the presence of the pressure exerted by the circulating blood. If this view is correct, one should expect that administration of the vitamin would among other things restore in considerable measure the lost tone, with the result that the right side of the heart would show a prompt return to normal size. It is obvious that this cannot be the complete explanation because much of the enlargement of the right side of the heart involves the right ventricle as well as the auricles.

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[25] Bliss, Sidney, and Green, Fred: Refection in the Rat, J. Nutrition **11:** 1-19 (Jan.) 1936.

[26] Birch, T. W., and Harris, L. J.: Bradycardia in the Vitamin B_1-Deficient Rat and Its Use in Vitamin B_1 Determinations, Biochem. J. **28:** 602-621, 1934.

[27] Shimazono, J.: B-Avitaminosis und Beriberi, Ergeb. d. inn. Med. u. Kinderh. **39:** 1-68, 1931.

[28] Aalsmeer, W. C., and Wenckebach, K. F.: Herz und Kreislauf bei der Beriberi-Krankheit, Berlin, Urban & Schwarzenberg, 1928.

VITAMIN B_1 AND THE NERVOUS SYSTEM

The pathology of vitamin B_1 deficiency is treated elsewhere and therefore will not be discussed here. Let it suffice to remind the reader that polyneuritis has always been one of the striking features of beriberi in human beings and of the advanced stages of experimental vitamin B_1 deficiency. This fact has led many clinicians to test the efficacy of this vitamin in treating various conditions in which polyneuritis is a feature of the syndrome. It has been possible to show that the polyneuritis exhibited by many alcohol addicts[29] is to be attributed to lack of vitamin B_1 rather than to a specific toxic action of the alcohol on nervous tissue. Likewise, many patients with polyneuritis associated with pregnancy[30] and various clinical conditions[31] have responded favorably to vitamin B_1 therapy, which suggests that under the clinical conditions prevailing in the United States certain of the effects of vitamin B_1 deficiency have been occurring without proper recognition by the clinician. Obviously such observations as these are of great importance in relation to the question of the adequacy of the supply of vitamin B_1 to these classes of patients.

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[29] Minot, G. R.; Strauss, M. B., and Cobb, Stanley: Alcoholic Neuritis: Dietary Deficiency as a Factor in Its Production, New England J. Med. **208**: 1244-1249 (June 15) 1933. Jolliffe, Norman, and Joffe, P. M.: Relation of Vitamin B (B_1) Intake to Neurological Changes in the Alcohol Addict, Proc. Soc. Exper. Biol. & Med. **32**: 1161-1162 (April) 1935. Jolliffe, Norman; Colbert, C. N., and Joffe, P. M.: Observations on the Etiologic Relationship of Vitamin B (B_1) to Polyneuritis in the Alcohol Addict, Am. J. M. Sc. **191**: 515-526 (April) 1936.

Administration of the vitamin to patients with pernicious anemia,[32] however, has had no effect on the nervous features of the disease which were due to involvement of the cord, although there was a more generalized improvement, evidenced by the patient's sense of well being and interest in food. If it is assumed that the damage to the spinal cord in these cases was irreparable, such a result is readily explained. Owing to the conflicting views[33] concerning the development of lesions of the cord as a result of specific vitamin B_1 deficiency, it is possible to take the view that administration of the vitamin should not be expected to improve the neurologic picture characteristic of pernicious anemia. Of course the patients may have been receiving a suboptimal supply of the vitamin, in which case administration of this dietary factor should result in a general improvement, which would be difficult, however, to define in precise terms.

The rotational movements frequently exhibited by animals suffering from specific vitamin B_1 deficiency suggest some lesions in the vestibular nuclei. Church[34] has offered evidence in support of this view. He has reported the discovery in the rat of perivascular hemorrhages in this region. The fact that these movements are not seen in all experimental animals suggests that the causative lesions in the brain are not of universal occurrence but instead are secondary to more fundamental tissue changes resulting from specific lack of vitamin B_1.

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[30] Plass, E. D., and Mengert, W. F.: Gestational Polyneuritis, J. A. M. A. **101:** 2020-2022 (Dec. 23) 1933. Strauss, M. B., and MacDonald, W. J.: Polyneuritis of Pregnancy, a Dietary Deficiency Disorder, J. A. M. A. **100:** 1320-1323 (April 29) 1933.

[31] Wilbur, D. L., and Snell, A. M.: Deficiency States Associated with Gastro-Intestinal Disease, Tr. Am. Gastro-Enterol. A., to be published.

[32] Fouts, P. J.: Kempf, G. F.; Greene, J. A., and Zerfas, L. G.: Vitamin B Intravenously in Treatment of Neurological Changes in Pernicious Anemia, J. Indiana M. A. **25:** 448-451 (Oct.) 1932.

[33] Gildea, E. F.; Kattwinkel, E. E., and Castle, W. B.: Experimental Combined System Disease, New England J. Med. **202:** 523-527 (March 13) 1930. Zimmerman, H. M., and Burack, Ethel: Lesions of the Nervous System Resulting from Deficiency of the Vitamin B Complex, Arch. Path. **13:** 207-232 (Feb.) 1932.

[34] Church, C. F.: Functional Studies of the Nervous System in Experimental Beriberi, Am. J. Physiol. **111:** 660-680 (April) 1935.

Church[35] has also suggested a method of vitamin B_1 assay based on his observation that B_1-deficient rats show a prolonged duration of rotary nystagmus. Although this may be true for the rat it does not appear to be the case with the dog according to my own experiments. In the latter species marked changes in the nystagmus were noticed only in the most advanced stage of B_1 deficiency and then not uniformly in all animals. Evidently there is again a failure of all species to show the same response to lack of this dietary factor. Molitor and Sampson[16] have extended this type of study to observations of the effect of large doses of the pure vitamin on the duration of nystagmus in normal rats and have observed no change.

VITAMIN B_1 AND METABOLISM

There is a wealth of evidence showing that vitamin B_1 has some role in the metabolic processes of the body. It has been shown by several investigators[36] that in the state of heightened metabolism, such as is produced experimentally by administration of thyroid, the organism's need for this dietary essential is increased. Such experiments, of course, do not indicate precisely what chemical processes in the body involve this vitamin.

In his early work on this problem Funk[37] suggested that vitamin B_1 has some role in the metabolic transformation of carbohydrate in the organism. Numerous later investigators have inclined to this view. The problem has been studied in several ways. Experimental animals have been allowed to subsist on B_1-deficient diets differing markedly in content of carbohydrate, protein and fat and the time required for development of the symptoms of B_1 deficiency taken as an indication of the relation of vitamin B_1 to the metabolism of one or more of these energy-yielding foodstuffs. There appears to be little doubt that a high carbohydrate diet is much more effective in producing the symptoms of B_1 avitaminosis than one high in fat. Out of this work has come considerable discussion of the vitamin B_1-sparing action of fat. A recent example of this kind of research is seen in the paper by Westenbrink[38] in which it is reported that pigeons on a high carbohydrate diet devoid of fat had typical symptoms of B_1 deficiency in nineteen (± 4) days, whereas another group subsisting on a high fat, carbohydrate-free ration required twenty-seven (± 7) days for the symptoms to appear.

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[35] Suggested to the United States Pharmacopeia Vitamin Conference, Aug. 13, 1934.

[36] Himwich, H. E.; Goldfarb, Walter, and Cowgill, G. R.: Studies in the Physiology of Vitamins: XVII. The Effect of Thyroid Administration upon the Anorexia Characteristic of Lack of Undifferentiated Vitamin B, Am. J. Physiol. **99:** 689-695 (Feb.) 1932. Cowgill, G. R., and Palmieri, M. L.: Studies in the Physiology of Vitamins: XXII. The Effect of Experimentally Induced Hyperthyroidism on the Vitamin B Requirement of Pigeons. Am. J. Physiol. **105:** 146-150 (July) 1933. Sure, Barnett, and Smith, Margaret E.: Hyperthyroidism and Nutrition: I. Vitamin B and Thyroxin, J. Nutrition **7:** 547-555 (May) 1934.

[37] Funk, Casimir: Studien über Beriberi: XI. Die Rolle der Vitamine beim Kohlenhydratstoffwechsel, Ztschr. f. physiol. Chem. **89:** 378-380, 1914.

Kemmerer and Steenbock[39] endeavored to answer experimentally the question whether this B_1-sparing action of fat is due to a greater conservation of the vitamin in the tissues. Various tissues of animals subsisting on suitable experimental rations were examined for their content of the vitamin. It was found that, when a diet low in vitamin B_1 and high in fat was fed, the tissues did not contain any more vitamin than when a diet low in vitamin and also low in fat was used. From such results it would appear that one cannot explain the vitamin B_1-sparing action of fat on the basis of greater conservation of the store in the tissue of this dietary essential.

Evans and his associates[40] were able to list various natural fats according to their ability to spare vitamin B_1 and offered the suggestion that the chemical nature of the fat in some way influences its action in this regard. This idea has received considerable support from the recent work of Salmon and Goodman.[13] Like Evans and his co-workers, these investigators found that coconut fat is the most effective natural fat of those tested. More striking, however, was the observation that spastic experimental beriberi in the rat can be cured by the feeding of glyceryl caprylate or caproate. "The effectiveness of esters of single fatty acids in alleviating the symptoms of vitamin B_1 deficiency in rats depended upon the length of the carbon chain of the fatty acid. The effectiveness was maximum at the 8-carbon acid and decreased in each direction from this point." When fats and single acid esters were given with a B_1-deficient diet, the apparent nutritive value was not the same as when the diet contained adequate amounts of the vitamin.

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[38] Westenbrink, H. G. K.: Vitamin B-Sparing Action of Fat, Acta brev. Neerland. **3:** 95, 1933; abstr. Chem. Abstr. **28:** 7319, 1934.

[39] Kemmerer, A. R., and Steenbock, Harry: A Study of the Sparing Action of Fats on the Vitamin B Content of Animal Tissues, J. Biol. Chem. **103:** 353-362 (Dec.) 1933.

[40] Evans, H. M.; Lepkovsky, Samuel, and Murphy, Elizabeth A.: The Sparing Action of Fat on Vitamin B: VII. The Effectiveness of Various Natural Fats in Sparing Vitamin B, J. Biol. Chem. **107:** 429-437 (Nov.) 1934.

The studies along this line appear to justify the conclusion that the function of vitamin B_1 is more likely related to carbohydrate than to fat metabolism, but the exact nature of the relationship is not evident and probably cannot be revealed by this type of experimentation. Westenbrink[41] has suggested three possible explanations: 1. When fat is fed, the organism uses in its metabolism less vitamin B_1 than it does when carbohydrate is fed. This is the view favored by Evans and his associates. 2. Whether the diet is high carbohydrate or high fat, the vitamin is used at the same rate, but the presence of much fat in some unknown manner affects the time of onset of the polyneuritis. 3. Under the two sets of dietary conditions vitamin B_1 is used at the same rate, but when carbohydrate is metabolized a toxic metabolite arises which in the absence of the vitamin is not removed and which therefore induces the polyneuritis. It will be seen that the last hypothesis constitutes a revival of the old toxin theory, with the toxin arising as a result of a "perverted intermediary metabolism" of carbohydrates.

The search for a specific function for vitamin B_1 has led many investigators to study the ability of various tissues from B_1-deficient animals to utilize oxygen or to perform certain chemical transformations which normal tissues have been shown capable of producing and which, presumably, are normal processes of intermediary metabolism. Crystallizing out of extensive investigations of this type has come the theory of Peters and his associates that vitamin B_1 functions as a co-enzyme in the metabolism of carbohydrate, more specifically in the oxidative breakdown of pyruvic acid. For a summary of the work leading to this conclusion the reader should consult the paper by Peters.[42] From the foregoing statements it is evident that two of the earlier ideas concerning the function of vitamin B_1 are supported by this theory, those relating the vitamin to carbohydrate metabolism and to biologic oxidative mechanisms.

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[41] Westenbrink, H. G. K.: Ueber den Einflusz der Menge und der Zusammenstellung der Ernahrung auf die Folgen von Vitamin B_1 Mangel, Arch. néerl. de physiol. **19**: 94-115, 1934.

[42] Peters, R. A.: The Biochemical Lesion in Vitamin B_1 Deficiency, Lancet **1**: 1161 (May 23) 1936.

It has long been known that lactic acid is a normal intermediate metabolite in the metabolic breakdown of dextrose. Evidence has also been available in support of the view that the lactic acid loses hydrogen under the influence of a lactate dehydrogenase with the result that the three carbon atom ketonic acid, pyruvic acid, is produced. Peters and his associates have been able to secure evidence that the cells of certain parts of the brain of the B_1-deficient pigeon show a lowered respiration in the presence of dextrose, a condition remedied by the addition of pure vitamin B_1. This action of the vitamin, on analysis, proves to be definitely that of a catalyst. Further, analysis reveals that these B_1-deficient cells, when respiring in the presence of added lactate without vitamin B_1, show an increase of pyruvate, and this increase is reduced if the vitamin is added to the in vitro system. Evidently, then, vitamin B_1 has some part in causing the disappearance of pyruvate. Study of the ratio of the extra oxygen taken up to the pyruvic acid disappearing on addition of the vitamin has revealed a value close to that yielded by the cells of normal brain tissue. On such results as these rests the contention that vitamin B_1 functions in some way to bring about oxidation of pyruvic acid. If this is correct it is obvious that in association with vitamin B_1 deficiency there should be an accumulation of pyruvic acid in the blood, and quantitative examination of this fluid for pyruvate might be made the basis of a biochemical method for detection of a state of vitamin B_1 deficiency. Platt and Lu,[43] among others, have tested this idea in cases of beriberi and observed definite increases of bisulfite-binding substances (pyruvic acid chiefly) in the blood, urine and cerebrospinal fluid. The validity of this test for B_1 deficiency obviously depends in considerable degree on the specificity of the production of pyruvate; if it can occur in association with other conditions besides vitamin B_1 deficiency its value is lessened to that extent. The observations made in cases of other disease conditions by Johnson, Meiklejohn, Passmore and Thompson[44] favor the view that the accumulation of pyruvate in the blood, urine and cerebrospinal fluid is reasonably specific for vitamin B_1 deficiency. This conclusion receives further support in the work of Thompson and Johnson,[45] who found the blood of B_1-deficient pigeons and rats to contain abnormally large amounts of bisulfite-binding substances, probably entirely pyruvic acid; in cured animals the concentration of these substances in the blood was reduced to that found in the normal organism.

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[43] Platt, B. S., and Lu, G. D.: Chemical and Clinical Findings in Beriberi with Special Reference to Vitamin B_1 Deficiency, Quart. J. Med. 5: 355-373 (July) 1936.

It would take me too far afield to discuss carbohydrate metabolism in all its details. Let it suffice to consider the problem of the breakdown of pyruvic acid as it may be affected by vitamin B_1. Two mechanisms may be mentioned, one involving simple decarboxilation with formation of acetaldehyde and carbon dioxide and the other involving oxidation with the production of acetic acid and carbon dioxide. The first type of reaction may be brought about through the action of the enzyme carboxylase in yeast; the second chemical change occurs in the presence of a pyruvic acid oxidase. Lohmann and Schuster[46] have reported the successful isolation in pure form of the natural cocarboxylase from bottom yeast and the finding that it is essentially a diphosphoric ester of vitamin B_1. It is with particular interest therefore that the student of this problem reads the report of Stern and Hofer[47] of the synthesis of cocarboxylase from pure vitamin B_1, which synthesis has been confirmed by Lohmann,[48] who, by using pyrophosphorylchloride instead of the phosphorus oxychloride employed by Stern and Hofer, obtained much higher yields. By the work of Tauber[49] and Euler and Vestin[50] it has been shown that cocarboxylase can be produced also by enzymic synthesis. The formula of this compound, according to Lohmann, is given herewith.

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[44] Johnson, R. E.; Meiklejohn, A. P.; Passmore, Reginald, and Thompson, R. H. S.: A Note on the Level of Carbonyl Compounds in Human Blood, Biochem. J. **29**: 2506-2509, 1935.

[45] Thompson, R. H. S., and Johnson, R. E.: Blood Pyruvate in Vitamin B_1 Deficiency, Biochem. J. **29**: 694-700, 1935.

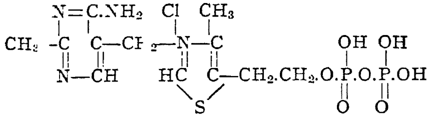
[46] Lohmann, K., and Schuster, P.: Ueber die Co-Carboxylase, Naturwissenschaften **25**: 26, 1937.

[47] Stern, K. G., and Hofer, J. W.: Synthesis of Co-Carboxylase from Vitamin B_1, Science **85**: 483-484 (May 14) 1937; Enzymologia **3**: 82-95, 1937.

[48] Private communication to K. G. Stern.

[49] Tauber, Henry: Enzymic Synthesis of Co-Carboxylase, Science **86**: 180 (Aug. 20) 1937.

[50] Euler, H. V., and Vestin, R.: Enzymatische Synthese von Cocarboxylase mit Vitamin B_1 und Phosphat, Naturwissenschaften **25**: 416, 1937.



Formula for cocarboxylase according to Lohmann.

There are many reasons for believing that the breakdown of pyruvic acid by decarboxylation is common in plant metabolism; whether it also occurs to a large extent in animal metabolism may be questioned. It is believed by many students of this subject that the second type of breakdown mentioned, namely, oxidation with production of acetic acid and carbon dioxide, is more likely in the animal organism. If vitamin B_1 assists in this reaction it functions also as a co-oxidase in the oxidation of pyruvic acid. Lipmann[51] has reported that such an action is exerted by Lohmann's pure cocarboxylase. Thus it appears that vitamin B_1, after phosphorylation in tissues to form a pyrophosphoric ester, can act as a coferment, assisting either the pyruvic acid carboxylase or its oxidase in breaking down this metabolite. Lipmann[51] suggested that one now has an explanation of the in vitro experiments of Peters, in which it appeared that after the addition of vitamin B_1 to the brain tissue, a substance x was converted into y, y being the true activator of pyruvic acid oxidation. It is suggested that the x of Peters is vitamin B_1 itself and the y the vitamin pyrophosphate into which it is converted by the tissue.[52]

This suggestion by no means constitutes a solution of the problem of pyruvic acid breakdown as it occurs in animal tissue, particularly the brain. Peters[53] has reported observations suggesting that still other factors besides vitamin B_1 (thiamin) must be involved. For example, Lohmann's pure cocarboxylase (vitamin B_1 pyrophosphate) is much less effective than vitamin B_1 itself in catalysing the oxygen uptake of avitaminous pigeon's brain tissue. This appears to be true even though the vitamin is found to phosphorylate rapidly under the conditions of the tissue experiment. Therefore one is justified, for the time being, in reserving judgment concerning the validity of a general application of the Lohmann yeast co-carboxylase hypothesis to animal metabolism.

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[51] Lipmann, Fritz: Pyruvic Acid Dehydrogenation, Vitamin B_1 and Cocarboxylase, Nature **140:** 25 (July 3) 1937.

[52] A more extended discussion of this subject was given by Williams, R. R.: The Chemistry of Thiamin (Vitamin B_1), J. A. M. A. **110:** 727 (March 5) 1938.

[53] Peters, R. A.: Pyruvate Oxidase in Brain IV. Co-carboxylase, Biochem. J. **30:** 2240-2246 (Dec.) 1937.

Another fact of interest in this connection is that reported by Peters[53] that "α-ketobutyric acid requires co-carboxylase for its decarboxylation by yeast, but it does not act as a substrate for the catatorulin effect (increased oxygen uptake upon addition of vitamin B_1)." Lest the reader be tempted to generalize from this to the belief that vitamin B_1 is involved in the breakdown of keto-acids or α-ketoacids in general in animal metabolism, his attention is called to the negative results reported by McGowan and Peters[54] in experiments with α-ketoglutaric and α-ketoadipic acids.

In the discussion of the vitamin B_1-sparing action of fat, reference was made to Westenbrink's suggestion of the possibility that a toxic intermediary metabolite may be responsible for the pathologic changes characteristic of vitamin B_1 deficiency. In the light of the work just reviewed, calling attention to lactic and pyruvic acids as metabolites important in this connection, one might consider that one or the other or both of these acids can be toxic and therefore accumulation beyond a certain point be productive of injury to tissues. However, it has not been possible to induce symptoms by injections of lactate.[26] Therefore, there is little support for the view that lactic acid is the hypothetic toxic metabolite. With respect to pyruvic acid the amounts present in the blood even under conditions of accumulation have proved to be small. It is unlikely therefore that this substance can be the searched-for toxin. Peters preferred to attribute to the deficiency of vitamin an inability of cells to develop from carbohydrate sufficient energy for normal cell processes, with a resultant failure of important functions, the precise nature of the functional derangement depending on the cells involved. According to this idea it is unnecessary to postulate the presence of a toxic metabolite.

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[54] McGowan, G. J., and Peters, R. A.: Pyruvate Oxidation in Brain III. Nature, Specificity and Course of Oxidation Catalysed by Vitamin B_1, Biochem. J. **31:** 1637-1641 (Sept.) 1937.

It will be noticed that this role in carbohydrate metabolism gives vitamin B_1 presumably a more or less generalized function in the body, instead of one peculiar to a given organ or group of cells. Also vitamin B_1 is placed in the category of substances involved in biologic oxidations and reductions, a group already containing numerous compounds. In view of this it may not be amiss to suggest the wisdom of suspending final judgment for the time being with respect to the universal character of the function of vitamin B_1 in all cells. May it not be that in some cells of the body the breakdown of pyruvic acid is accomplished by mechanisms other than the ones already demonstrated to involve vitamin B_1?

PHARMACOLOGY OF VITAMIN B_1

The isolation of vitamin B_1 and its synthesis have made possible the study of its pharmacologic action. Molitor and Sampson [16] have conducted such an investigation, some of the results of which have already been cited. An additional conclusion of interest is that pure vitamin B_1 increases the oxygen consumption only in the B_1-deficient animal. Relatively enormous doses are without effect in the normal organism. All the pharmacologic tests of this substance yielded essentially the same conclusion: Vitamin B_1 exerts a demonstrable action only in the B_1-deficient animal. As no toxic symptoms of any kind were observed when doses approximating 25,000 or more times the estimated daily requirement were administered, it is evident that this dietary essential now available in pure form for clinical use is not to be regarded as a drug physiologically active within a certain range of dosage and dangerous to use when this range is exceeded. Instead, it is to be considered a normal and necessary part of the organism which exerts its function whenever it is needed, and any excess of supply beyond a modest amount capable of storage in the tissues for a relatively short period is eliminated through the kidneys. It is readily absorbed whether given by mouth or parenterally. With this vitamin now available in pure form and methods being perfected for its chemical determination, it is not unlikely that the future will soon see the abandonment of various "units" by which to express its activity, in favor of the use of definite dosages of the pure substance.

CHAPTER IX

THE PATHOLOGY OF BERIBERI

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Beriberi is a disease resulting from faulty metabolism and is directly caused by deficiency of the antineuritic vitamin B_1 and possibly other deficiencies in the diet. Among Orientals, this deficiency is usually produced by the too exclusive use of decorticated or polished rice; but it may equally be caused by the too exclusive use of white wheat flour and other carbohydrate staples. Clinically, beriberi is characterized by degenerative changes in the nervous system including a multiple peripheral neuritis, which may exist alone but is often combined with generalized edema and serous effusions, and by a tendency to the development of cardiac hypertrophy, which frequently results in cardiac failure and sudden death. In this definition vitamin B_1, as distinguished from other fractions of the vitamin B complex, is the compound isolated and synthesized by R. R. Williams, a compound of pyrimidine and thiazole which has been designated by Williams as thiamin.

As is evident from the foregoing definition, the pathology of beriberi may be discussed under three heads: cardiac pathology, degenerative changes in the nervous system, and anasarca. While much has been learned concerning the etiology of beriberi from animal experimentation, one should distinguish between the pathology of human beriberi and that of birds and animals.

THE PATHOLOGY OF HUMAN BERIBERI

Knowledge of human pathology is more limited than it should be after so many years of study, but it is significant. Death does not appear to be caused by neuritis, however extensive it may be, but by cardiac hypertrophy followed by sudden dilatation and cardiac failure. Accordingly, on postmortem examination the heart is

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found markedly dilated and hypertrophied especially on the right side, the chambers being filled with blood not very firmly clotted. The valves are normal, nor is there any obvious degeneration. Ellis[1] in 1898 in 125 necropsies of beriberi found the average weight of the heart to be 13.37 ounces (379 Gm.), the right side in nearly every case being much enlarged. During the same period 204 hearts from patients dying of other diseases averaged just under 9 ounces (255 Gm.). McLaughlin and Andrews[2] described finding the same conditions in infantile beriberi. "Probably the most striking and constant change is found in the right heart. Its musculature is coarse and firm and forms much the larger part of the organ, even in the contour of the apex. Its trabeculae and papillary muscles are prominent, while its cavity is enlarged. The wall of the right ventricle may measure from 5-7 millimeters in thickness while the left measures only 3-5 millimeters." The weight of the heart in Andrews' cases of infantile beriberi averaged 34.1 Gm. with a maximum of 54 Gm., while in death from other diseases the average heart weight was 20 Gm., with a maximum of 32 Gm. Later figures[3] are consistent. The average weight among Japanese beriberi patients has been given as 368 Gm., while the maximum of normal hearts is 300 Gm. The enlargement is most pronounced in the right chambers, and the left side of the heart may be very small. The right auricle is huge, with a paper thin wall through which the dark blood within may be seen. The evidence indicates that there is a generalized arteriolar or capillary dilatation. This results in an increased flow of blood to the right side of the heart, which first hypertrophies and later dilates.

As the result of the back pressure caused by the failure of the right side of the heart, pulmonary edema occurs in at least half of the cases, and chronic passive congestion of the liver, spleen, kidneys and intestine is constant. Punctate hemorrhages are often found subpleurally as well as in the stomach and duodenum, while the vessels in the walls of the stomach and intestine are dilated and hyperemic. The liver often has the typical nutmeg character, the spleen is perceptibly increased and hyperemic, and the kidneys are cyanotic. Microscopically, some degree of cloudy swelling or fatty degeneration may be found in the liver and kidneys in some cases. Fragmentation of the heart muscle has been described, but these changes are now generally believed to be merely post mortem. One of the obvious difficulties has been the rapid change occurring in the tropics, where most of these cases have been studied.

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[1] Ellis, W. G.: A Contribution to the Pathology of Beriberi, Lancet **2**: 958, 1898.

[2] McLaughlin and Andrews: Studies on Infantile Mortality, Philippine J. Sc. **5**: 159, 1910.

[3] Eddy, W. H., and Dalldorf, Gilbert: The Avitaminoses, Baltimore. Williams & Wilkins Company, 1937, p. 100.

The cause of the hypertrophy and dilatation of the right side of the heart is not known. Formerly attributed to degeneration of the pneumogastric nerves, it is only necessary to state that this explanation does not account for the fact that the right side is usually solely affected, and there is apparently no correlation between the intensity of degeneration of these nerves and the cardiac disturbance. Wenckebach[4] explained the combination of cardiac hypertrophy and weakness by assuming that the hypertrophy was caused neither by an increased number of muscular fibers nor by a true increase in their size, but that this increase in size was caused by the imbibition of fluid. He pointed out that the electrocardiogram was normal and that when vitamin B extracts were administered the hypertrophy melted away "like ice in the sun." It is also true that cardiac hypertrophy is much more frequent in the "wet" than in the "dry" forms of beriberi. Unfortunately this explanation does not account for the peculiar selection of the right side of the heart for this action, nor has it been proved, which should be a simple matter. If the hypertrophy is caused simply by the imbibition of fluid by the muscle fibers, this could be shown by comparing the weight of this dried heart muscle with the dried weight of normal hearts. This has not been done for human cases. But Newcomb[5] examined pigeons' hearts. Of ninety-six pigeons fed on deficient diets, eighty-four developed polyneuritis columbarum, eight developed beriberi columbarum, which by definition included edema and enlarged hearts in addition to polyneuritis, and four died from other causes. The heart muscle of all these birds was dried and weighed. Newcomb concluded that "The beriberi pigeons do not show a greater water content in their heart muscle than other pigeons fed on these diets. If a large heart in these birds is to be explained by water retention, they should, and quite definitely do not."

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[4] Wenckebach, K. F.: Heart and Circulation in a Tropical Avitaminosis (Beriberi), Lancet **2**: 265 (Aug. 11) 1928. Alsmeer and Wenckebach: Herz und Kreislauf bei der Beriberi-Krankheit, Berlin, Urban & Schwarzenberg, 1929.

[5] Newcomb, C.: Water Content of Heart Muscle in Beriberi Columbarum, Indian J. M. Res. **17**: 721 (Jan.) 1930.

Weiss and Wilkins[5a] studied a number of cases of cardiovascular diseases in Boston, believed to be caused by deficiency of B_1 because the symptoms disappeared after administration of that vitamin, while in patients with cardiac conditions not due to B_1 deficiency, large doses of crystalline B_1 produced no effect. In the deficient cases when death was due to sudden circulatory collapse, the myocardial fibers and the conductive bundle exhibited various degrees of hydropic degeneration together with intercellular edema and collagen. These are the same microscopic findings described by Wenckebach; but in this group of cases hypertrophy and dilatation of the right ventricle was neither so marked or so uniform as has been observed in the hearts from cases of Oriental beriberi. Further studies of the microscopic pathology of the typical right-sided cardiac enlargement as it occurs among Oriental rice eaters are required.

NERVE DEGENERATION

The Spinal Cord.—The changes in the cord are not usually apparent grossly, but Bentley[6] reported nineteen necropsies and found the brain and its membranes congested, and the spinal cord congested and softened. "The lesions of the cord were so gross as not to require the use of the microscope to see them, for in some, only a few hours after death, the cord was soft and diffluent with hemorrhages, marked congestion and extensive edema quite sufficient in themselves to cause all the symptoms. My observations go to prove that it is a cord lesion which involves both sensory and motor functions of central origin, a subacute inflammation of the spinal cord and its membranes." While most observers have not found such gross changes or have attributed them to postmortem degeneration, there can be no doubt as to the microscopic changes, which have been reported by all observers. Degeneration of the medullary sheath has been demonstrated in scattered fibers in all tracts of the cord, but especially in the posterior columns as well as in both anterior and posterior nerve roots. In some cases the axis cylinder is fragmented. Degeneration of the medullary sheath is best demonstrated by the Marchi method, of the cells by Nissl or Giemsa stains. The axis cylinder is readily seen when degeneration of the medullary sheath is extensive but may be brought out more clearly by special stains, such as those of Golgi or Mallory.

[5a] Weiss and Wilkins: The Nature of the Cardiovascular Disturbances in Nutritional Deficiency States (Beriberi), Annals of Internal Medicine, **11:** 104 (July) 1937.

[6] Bentley, F. J.: Beriberi, Its Etiology, Symptoms, Treatment and Pathology, Edinburgh, Y. J. Pentland, 1893.

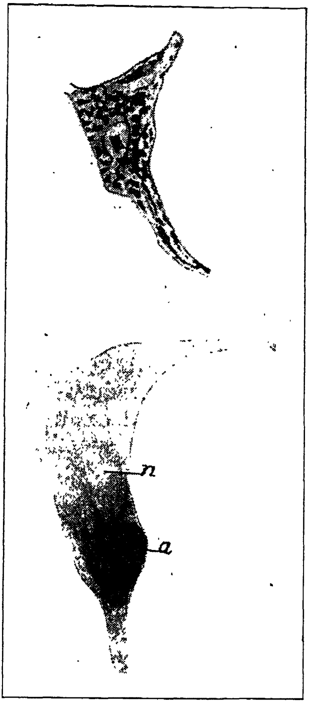

Fig. 1.—Nerve cells from the lumbar region of fowls stained by Giemsa. Upper cell, normal, showing tigroid bodies. Lower from fowl suffering from polyneuritis gallinarum, showing disappearance of tigroid bodies, collection of stainable material in one mass, and degeneration of nucleus.

Wright[7] found changes in the posterior spinal ganglion and anterior horn cells, and in the nuclei of the medulla in cases in which the fibers originating from these cells were atrophied. That is, atrophy or loss of function in the axon leads to or is concomitant with disturbances in its trophic cell. The changes found in these cells depend in intensity on the length of time the polyneuritis has existed and include swollen and dislocated nuclei and loss of the Nissl bodies, which break down into a powdery mass. In more advanced degeneration this powdery mass almost disappears and there is vacuolation of the cell with rupture of its membrane and fragmentation of its processes. Similar changes have been found in the ganglion cells of the medulla and pons. These degenerative changes in most cases are not to be regarded as complete, which would mean the death of the cell, from which there could be no recovery; but they do indicate a very complete exhaustion of the affected cells.

The Peripheral Nerves.—Since the legs are first affected in beriberi, degeneration is most marked in the sciatic nerves and its branches, but some degree of degeneration may be found in any peripheral nerve. Degeneration of the myelin sheath is constant and affects the majority of the fibers, the myelin being broken up into balls or beads and eventually disappearing. When this occurs, the axis cylinders may show a coiled appearance and in certain cases are fragmented or atrophied. The more chronic and advanced the case, the more nerve cylinders are affected, but usually the majority appear normal even when the medullary sheath shows advanced degeneration. At the same time that such advanced changes are found in the nerves supplying the legs, the brachial nerves may show only a diffuse blackening of the myelin sheath with the Marchi stain. Similar degenerative changes have been found in the cranial nerves, particularly the phrenics and the vagi. It is especially to be noted that degeneration of the sympathetic system has been demonstrated, as in branches of the cardiac plexus, the splanchnic nerves and branches of the solar and renal plexuses.

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7. Wright, H.: Changes in the Neuronal Centers in Beriberic Neuritis, Brit. M. J. **1**: 1610, 1901; An Inquiry into the Etiology and Pathology of Beriberi, Studies from the Institute of Medical Research Federated Malay States, 1902.

The muscles supplied, particularly of the leg and thigh, are markedly atrophied, with loss of cross striation and shrinkage of the sarcoplasm, often combined with cloudy swelling or fatty degeneration. These changes are not characteristic of beriberi but may be found in the muscles in any variety of polyneuritis. In very advanced cases, the muscles of the arms or even of the trunk are similarly atrophied, and such muscles are exceedingly tender to the touch, causing the patient to flinch from the gentlest examination.

EDEMA AND SEROUS EFFUSIONS

Dry beriberi was described by Bontius in 1642 and edema and serous effusions were not described until 1808, and in 1812 Marshall described dry beriberi and wet beriberi as two separate diseases. In 1835 Malcolmson pointed out that cases commencing as wet beriberi became transformed into dry beriberi after a sudden diuresis, while edema often developed in dry cases. From this he concluded that dry and wet cases were but two types of the same disease. The proportion of wet cases will therefore vary in different outbreaks of the disease.

When edema occurs, it is noticed first in the legs and thighs, which often swell to such an extent as to make walking difficult. While often confined to these limbs it may become general, although the face is rarely affected. Edema of the lungs may be found in 50 per cent of the cases.

On making the postmortem incision in such cases, fluid blood exudes from the cut vessels, and serous fluid in the subcutaneous tissue is at once noticed. With regard to effusions, the pericardium is most frequently affected, followed by the pleura and the peritoneum. The effused fluid is clear and of a distinct greenish yellow.

In sixty-four cases in Java, pericardial fluid was found in sixty-two, hydrothorax in fourteen, and ascites in nine.

In 125 cases from the Malay States, hydrops of the pericardium was found in eighty-one, pulmonary edema in seventy-eight, hydrothorax in ten, and ascites in five. The amount of fluid in the pericardium varies from 50 to 500 cc.; in hydrothorax from 50 to 2,000 cc. The amount of fluid in the peritoneum is seldom large enough to cause much distention.

PERCENTAGE OF WET CASES IN INFANTILE BERIBERI

Cases of dry beriberi in infants have been described, but they are relatively uncommon; in fact, McLaughlin and Andrews,[2] studying 219 infants dying under 1 year of age, showed that 124 of them died of a condition which they designated as moist beriberi. In later necropsies, Andrews[8] based his diagnosis on four points: (1) cardiac hypertrophy and dilatation, (2) congestion of the viscera, (3) anasarca and (4) absence of any other condition to account for death. All the cases of infantile beriberi identified by postmortem examination were therefore wet beriberi.

The cause of this general anasarca is not known. The fluid has the appearance and characteristics of simple transudates and has been attributed to degeneration of the sympathetic system and to the back pressure from the functional impairment of the right side of the heart, and more recently Eppinger[9] has considered the fluid to be the product of serous inflammation. Neither of these explanations is satisfactory. The edema is more probably caused by generalized capillary dilatation, with increased permeability to plasma of a normal osmotic tension. It was early noted that the edema was often dissipated after a sudden diuresis. This was probably due to a sudden change in diet because the edema in cases of ship beriberi also cleared up miraculously as soon as the sailors reached land and ate shore food. Again, Chamberlain and Vedder[10] found that the edema of infantile beriberi was dissipated by diuresis in two or three days as the result of administration of an extract of rice polishings that were primarily extracted with 95 per cent alcohol. This extract contained no protein and did contain large amounts of vitamin B_1. But this extract contained also many other nitrogenous bases. As the result of these considerations it is possible that the edema is caused by the deficiency of some as yet unidentified food constituent, possibly some fraction of the B complex aside from B_1, or some purine or other organic base. On the other hand the observations of Weiss and Wilkins [5a] indicate that the edema clears up after administration of crystalline B_1. Further observations at this point are needed.

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[8] Andrews, V. L.: Infantile Beriberi, Philippine J. Sc. **7**: 67, 1912.

[9] Eppinger, Hans: Die Serosa Entzündung, Vienna, Julius Springer, 1935.

[10] Chamberlain, W. P., and Vedder, E. B.: The Cure of Infantile Beriberi by the Administration of an Extract of Rice Polishings, Bull. Manila M. Soc. **4**: 26, 1912. Albert, Jose: Studies on Infantile Beriberi Based on Five Hundred and Fourteen Cases, Philippine J. Sc. **45**: 297 (June) 1931.

BERIBERI IN BIRDS AND ANIMALS

Are the degenerative changes in the nervous system found in birds and rats fed on B_1 deficient diets due to beriberi or to inanition? Eijkman, who first discovered polyneuritis gallinarum, and most subsequent observers have considered the disease analogous to beriberi in man, but in recent years there has been a strong tendency to ascribe most of these pathologic changes to inanition caused by the failure of birds or animals to eat. It is well recognized that B_1 deficient diets cause an early failure of appetite. As early as 1911 Chamberlain, Bloombergh and Kilbourne [11] found that fowls

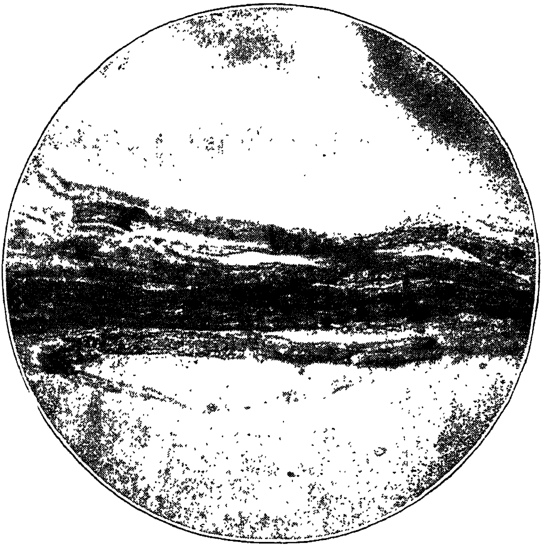

Fig. 2.—Nerve fibers from a rat starved to death, but receiving 3 milligrams of thiamin daily. Marchi method. There is no difference in degeneration in this case as compared with nerves in starvation without thiamin.

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[11] Chamberlain, W. P.; Bloombergh, H. D., and Kilbourne, E. D.: A Study of the Influence of Rice Diet and of Inanition on the Production of Multiple Neuritis in Fowls, Philippine J. Sc. **6**: 177, 1911.

starved to death developed a degeneration of the peripheral nerves not readily distinguished from the degeneration found in polyneuritis gallinarum induced by an exclusive rice diet. Quite recently, Davison and Stone[12] starved rats and, finding pathologic evidences of polyneuritis, concluded that "the pathological changes in the nervous system in animals suffering from inanition are essentially the same as in those deprived of vitamin B_1 or of both vitamins B_1 and B_2. If anything the changes in the peripheral nerves in animals subjected to inanition are more marked."

Kon and Drummond[13] attempted to separate the effects of inanition from deficiency in vitamin B by feeding four groups of pigeons. The pigeons in lot 1 received an artificial ration deficient in B. The birds in lot 2 were forced to eat the same quantity of food eaten by the birds in lot 1 plus 1 Gm. of yeast extract (marmite). The pigeons in lots 3 and 4 were forcibly fed with amounts of the deficient ration calculated to maintain weight. The pigeons in lot 4 received in addition 1 Gm. of yeast extract. The authors attributed the nerve changes to inanition, chiefly because the birds in lots 1, 2 and 3 lost weight rapidly. But the birds in lot 4, supposed to receive an adequate diet, also lost weight, and all these birds in lots 1 and 3 fed on the deficient diet were deprived of the entire B complex instead of only B_1.

It must be admitted that rats starved to death show definite degenerative changes in the nerves of the same general character as those found in polyneuritis. Nor are these degenerative changes eliminated when such starving animals are given adequate amounts of B_1. The degenerative changes are as a rule quite slight and consist of occasional minute droplets taking the black stain and according to my belief are not as extensive as the changes found in polyneuritis caused by feeding diets deficient in B_1.

Admitting that birds and rats fed adequate rations except for B_1 lose weight, and that starving rats given adequate amounts of B_1 show degenerative changes in their nerves, there is an excellent reason for not attributing these changes to inanition, for this is too simple an explanation, or, rather, it is an explanation that explains nothing. The nerve changes occurring in polyneuritis gallinarum are precisely similar to those occurring in human beriberi, and human patients are often well nourished and do not lose weight. Actually, one of the reasons often urged against dietary deficiency as the cause of human beriberi has been the fact that many patients maintain their weight and appear well nourished. Further studies on the physiologic action of B_1 may elucidate these difficulties.

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[12] Davison, Charles, and Stone, Leo: Lesions of the Nervous System of the Rat in Vitamin B Deficiency, Arch. Path. **23:** 207 (Feb.) 1937.

[13] Kon, S. K., and Drummond, J. C.: Physiological Action of Vitamin B: Study of Vitamin B Deficiency in Pigeons, Biochem. J. **21:** 632, 1927.

Birds fed diets deficient in B_1 ordinarily develop only polyneuritis, comparable to dry beriberi in man. But McCarrison[14] succeeded in producing edema and enlarged hearts in a considerable number of pigeons, in addition to the polyneuritis, by feeding them on diets similar to those used by natives of India who develop beriberi. These diets included, in addition to rice, certain proportions of dal (a legume) and other antineuritic foods. McCarrison called such cases beriberi columbarum as distinguished from polyneuritis columbarum, the term applied to polyneuritis alone.

POLYNEURITIS GALLINARUM OR COLUMBARUM

The nerve lesions in such birds have been described by Vedder and Clark.[15] In sciatic nerves stained by the Marchi method, practically every fiber shows some evidence of degeneration, the extent of which varies greatly in the fibers of the same nerve. Some fibers show only a localized slight blackening, but the majority show more extensive change, frequent droplets of degenerating myelin. In from 10 to 15 per cent of the fibers, there is in addition a swelling of the nerve sheath at these points and a disintegration of the axis cylinder, which is fragmented.

The Stages of Myelin Degeneration.—The earliest stage consists of slight swelling of the medullary sheath and a tendency toward segmentation at the circumference. Then follows the stage in which the largest globules distending the nerve are seen. Next is a stage in which the globules are much smaller though quite black and which only partly fill the sheath of

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[14] McCarrison, Robertt: Beriberi Columbarum, India J. M. Res. Memoir 10, March 1928.

[15] Vedder, E. B., and Clark, E.: A Study of Polyneuritis Gallinarum Philippine J. Sc. **7**: 423 (Oct.) 1912.

Schwann. Then follows a stage in which the shrunken neurilemma contains only a few scattered droplets of fat.

The condition does not commence in peripheral branches and extend to the cord but appears to be as intense near the cord as in peripheral branches. Similar changes are found in the vagi in all cases as well as in the ventral and dorsal nerve roots but were much more advanced in the ventral roots.

The Spinal Cord: Degeneration was observed in the fiber tracts of all columns. Evidences of degenerated fibers were found in the thoracic region as well as in lower sections. The degenerative changes in the myelin sheath were the same as those described for the earlier changes in the nerves.

The Brain: Similar observations in Marchi preparations were made on the fiber tracts of the medulla, pons, midbrain and internal capsule, and degenerated fibers were found in each one of these brain divisions comparable to those found in the cord.

The Nerve Cells: Stained by the Nissl or Giemsa stains, changes are most marked in the cells of the lumbosacral region in both anterior and posterior horns of the cord. In these cells the stainable tigroid substance shows a marked tendency to break down into granular material diffused throughout the cell, or in later stages for the collection of this granular material at one side of the cell around the base of one of the processes. This granular appearance is suggestive of disintegration rather than solution of the tigroid substance. The cells of other parts of the gray matter of the cord do not show this change to such an extent, but they stain poorly and the cells have a pale appearance. In some instances the nuclei are dislocated or pale and poorly stained, suggesting degenerative change; but this was noted in very few cells. On the whole, the cells appear to indicate exhaustion rather than degeneration, and such changes have been brought about by fatigue. It appears therefore that, along with degeneration of the peripheral nerves and in the fiber tracts of the cord and brain, there occur changes in the nerve cells of the anterior and posterior horns of the spinal cord which may or may not signify actual degeneration, which probably does not occur until near the death of the fowl. Complete degeneration obviously cannot occur so long as recovery is possible. But it is recognized that in extreme cases of polyneuritis gallinarum recovery will not occur even after adequate administration of soluble B_1, and this may be due to actual degeneration of these nerve cells.

Comparing these changes with the changes in the nervous system already described as occurring in human beriberi, the great similarity of the two conditions is emphasized.

McCarrison[16] has described hypertrophy of the adrenal glands in polyneuritis of birds induced by B_1 deficient diets and an increased output of epinephrine. Several observers have confirmed this enlargement of the adrenals in birds, but Kon and Drummond[13] found no difference in the amount of epinephrine secreted by polyneuritic pigeons as compared with normal birds. This enlargement of the adrenals appears to be confined to birds, for according to my limited experience the adrenal glands are not enlarged in rats on B_1 deficient diets and, so far as I am aware, enlarged adrenals have not been mentioned either in animal experiments or in cases of human beriberi.

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[16] McCarrison, Robert: Studies in Deficiency Disease, Oxford Medical Publications, 1921.

Schwann. Then follows a stage in which the shrunken neurilemma contains only a few scattered droplets of fat.

The condition does not commence in peripheral branches and extend to the cord but appears to be as intense near the cord as in peripheral branches. Similar changes are found in the vagi in all cases as well as in the ventral and dorsal nerve roots but were much more advanced in the ventral roots.

The Spinal Cord: Degeneration was observed in the fiber tracts of all columns. Evidences of degenerated fibers were found in the thoracic region as well as in lower sections. The degenerative changes in the myelin sheath were the same as those described for the earlier changes in the nerves.

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16. McCarrison, Robert: Studies in Deficiency Disease, Oxford Medical Publications, 1921.

CHAPTER X

THE THERAPEUTIC USE OF VITAMIN B_1 IN POLYNEURITIS AND CARDIOVASCULAR CONDITIONS

CLINICAL INDICATIONS

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Vitamin B_1 deficiency in man involves predominantly the nervous and circulatory systems. Clinical descriptions of the disturbances in these systems under the name beriberi date back to ancient Chinese medical writings. Although known to be widespread in the Orient and common in Newfoundland, beriberi has been considered rare in the United States of America[1] except for its occasional occurrence in epidemic form in penal institutions and insane asylums. During the past decade, however, it has been shown that beriberi is and has been endemic in the United States in characteristic form.[2] Alcoholic polyneuritis, the toxic polyneuritis of pregnancy, diabetic, biliary and gastrogenous polyneuritides, postinfectious polyneuritis, the Korsakoff syndrome and other similarly misleading names have concealed the true diagnosis of vitamin B_1 deficiency in the Western World. Clinically and pathologically identical to the Oriental disease, these polyneuritides differ only in the particular mechanism

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[1] Cowgill, G. R.: Vitamin B_1 in Relation to the Clinic, J. A. M. A. **98:** 2282 (June 25) 1932.

[2] (*a*) Shattuck, G. C.: Relation of Beriberi to Polyneuritis from Other Causes, Am. J. Trop. Med. **8:** 539 (Nov.) 1928. (*b*) Strauss, M. B., and McDonald, W. J.: Polyneuritis of Pregnancy, a Dietary Deficiency Disorder, J. A. M. A. **100:** 1320 (April 29) 1933. (*c*) Wechsler, I. S.: Etiology of Polyneuritis, Arch. Neurol. & Psychiat. **29:** 813 (April) 1933. (*d*) Minot, G. R.; Strauss, M. B., and Cobb, Stanley: "Alcoholic" Polyneuritis; Dietary Deficiency as a Factor in Its Production, New England J. Med. **208:** 1244 (June 15) 1933. (*e*) Urmy, T. V.; Ragle, B. H.; Allen, A. W., and Jones, C. M.: Beriberi Secondary to Short-Circuited Small Intestine, New England J. Med. **210:** 251 (Feb. 1) 1934. (*f*) Strauss, M. B.: The Rôle of the Gastro-Intestinal Tract in Conditioning Deficiency Diseases, J. A. M. A. **103:** 1 (July 7) 1934. (*g*) Strauss, M. B.: The Etiology of "Alcoholic" Polyneuritis, Am. J. M. Sc. **189:** 378 (March) 1935. (*h*) Jolliffe, Norman; Colbert, C. N., and Joffe, P. M.: Observations of the Etiologic Relationship of Vitamin B (B_1) to Polyneuritis in the Alcohol Addict, ibid. **191:** 515 (April) 1936.

by which the deficiency is brought about. Cowgill[3] has shown that the vitamin B_1 requirement of man is directly proportional to body weight, caloric intake and metabolism. Beriberi develops in the Chinese coolie primarily because his customary diet, consisting largely of polished rice, contains an insufficient amount of vitamin B_1 per calory. Infections or excessive physical exertion, raising the total metabolism, not infrequently precipitate the acute symptoms of deficiency.[4]

Beriberi develops in the westerner because complicating factors render his vitamin B_1 intake inadequate. The person addicted to chronic alcoholism usually ingests a sufficient amount of vitamin B_1 for his caloric intake in the form of food[2h] but each calory received in the form of alcohol increases the total vitamin B_1 requirement. Furthermore, gastro-intestinal disturbances,[2d] not uncommon among steady drinkers, may so interfere with absorption[5] that the amount of vitamin B_1 absorbed is considerably reduced. Thus the chronic excessive imbiber is prone to develop B_1 deficiency. The pregnant woman, when nauseated, often restricts her diet to concentrated carbohydrate foods, low in vitamin B_1 content. Then, because of vomiting, she fails to retain all that she does ingest.[2b] Further, her metabolism, and with it her vitamin B_1 requirement, is elevated by gestation, and it is possible that her powers of assimilation are reduced.[6]

The patient with pyloric stenosis is prone to partake of a diet low in vitamin B_1 and usually loses part of this small amount through vomiting.[7] In colitis of various types, improper dietary regimens may lead to a limited intake of vitamin B_1 and there may well be little retained because of diarrhea and alteration of intestinal absorption.[8] Prolonged febrile illnesses are frequently associated with anorexia. Diets are limited. Fever raises the metabolic rate and with it the vitamin B_1 requirement. Further, fever may well interfere with the assimilation and utilization of vitamin B_1 as well as other substances derived from food. Cirrhosis and other diseases of the liver, arteriosclerosis and other conditions involving severe damage to vital organs may all raise the vitamin B_1 requirement by acting to inhibit the utilization of this substance. It is in such fashions that beriberi develops most commonly in the United States.

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[3] Cowgill, G. R.: The Vitamin B Requirement of Man, New Haven, Conn., Yale University Press, 1934.

[4] Keefer, C. S.: The Beriberi Heart, Arch. Int. Med. **45:** 1 (Jan.) 1930.

[5] Groen, J.: The Absorption of Glucose from the Small Intestine in Deficiency Disease, New England J. Med. **218:** 247 (Feb. 10) 1938.

[6] Strauss, M. B., and Castle, W. B.: Studies of Anemia in Pregnancy: I. Gastric Secretion in Pregnancy and the Puerperium, Am. J. M. Sc. **184:** 655 (Nov.) 1932.

[7] Duvernay: Névrites périphériques toxiques liées à de la rétention gastrique, Lyon méd. **109:** 53 (July 14) 1907. Couréménos and Conos: Polynévrité dans l'ulcère gastrique, Encéphale **7:** 423 (Nov. 10) 1909. Klippel, M., and Weil, M. P.: Les complications nerveuses de l'ulcère de l'estomac (polynévritiés et pseudotabes polynévritique), Presse méd. **19:** 753 (Sept. 23) 1911. Libert, E.: Stenose ulcereuse du pylore accompagnée de pseudotabes polynévritique chez un syphilitique ancien, Arch. d. mal. de l'app. digestif. **20:** 493 (April) 1930.

Of course, not all polyneuritides and circulatory disturbances of unknown etiology are due to vitamin B_1 deficiency. It is quite possible also that some of the supposed symptoms of B_1 deficiency in man are in reality due to some other factor closely associated with vitamin B_1 in nature, such as riboflavin, nicotinic acid. and other compounds. Vitamin B_1 was isolated only recently, and it is as yet too early to have tested the effect of pure vitamin B_1 on all of the conditions generally ascribed to vitamin B_1 deficiency and which are amenable to therapy with crude B_1 preparations. As to the clinical differentiation of the different types of polyneuritides, this may be aided materially by the history of dietary deficiencies or conditioning factors, such as alcoholism, pregnancy, diabetes and gastrointestinal disorders. Frequently, however, the differentiation can be made from the clinical examination of the patient.

NEURAL MANIFESTATIONS OF VITAMIN B_1 DEFICIENCY

The onset of vitamin B_1 deficiency may be sudden, but it is generally insidious. Heaviness of the legs and tenderness of the calf muscles when they are squeezed are usually the earliest manifestations. Walking short distances is unimpaired, but when longer walks are attempted weakness may become apparent. Frequently by which the deficiency is brought about. Cowgill[3] has shown that the vitamin B_1 requirement of man is directly proportional to body weight, caloric intake and metabolism. Beriberi develops in the Chinese coolie primarily because his customary diet, consisting largely of polished rice, contains an insufficient amount of vitamin B_1 per calory. Infections or excessive physical exertion, raising the total metabolism, not infrequently precipitate the acute symptoms of deficiency.[4]

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[8] Haas, S V.: Beriberi in Late Infancy: The Result of Celiac Disease, Arch. Pediat. **46:** 467 (Aug.) 1929. Keefer, C. S., and Yang, C. S.: The Treatment of Secondary Anemia, Arch. Int. Med. **48:** 537 (Oct.) 1931. Jones, C. M.: Peripheral Complications of Ulcerative Colitis, M. Clin. North America **16:** 919 (Jan.) 1933. Kohn, S.: Mitteilungen über einige seltenere Krankheitsfälle aus der privaten Praxis, Prager med. Wchnschr. **35:** 584, 1910. Viets, H. R., and Allen, A. W.: Unpublished observations quoted by Urmy, Ragle, Allen and Jones.[2e] Groen.[5]

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[7] Duvernay: Névrites périphériques toxiques liées à de la rétention gastrique, Lyon méd. **109:** 53 (July 14) 1907. Couréménos and Conos: Polynévrité dans l'ulcère gastrique, Encéphale **7:** 423 (Nov. 10) 1909. Klippel, M., and Weil, M. P.: Les complications nerveuses de l'ulcère de l'estomac (polynévritiés et pseudotabes polynévritique), Presse méd. **19:** 753 (Sept. 23) 1911. Libert, E.: Stenose ulcereuse du pylore accompagnée de pseudotabes polynévritique chez un syphilitique ancien, Arch. d. mal. de l'app. digestif. **20:** 493 (April) 1930.

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[8] Haas, S V.: Beriberi in Late Infancy: The Result of Celiac Disease, Arch. Pediat. **46**: 467 (Aug.) 1929. Keefer, C. S., and Yang, C. S.: The Treatment of Secondary Anemia, Arch. Int. Med. **48**: 537 (Oct.) 1931. Jones, C. M.: Peripheral Complications of Ulcerative Colitis, M. Clin. North America **16**: 919 (Jan.) 1933. Kohn, S.: Mitteilungen über einige seltenere Krankheitsfälle aus der privaten Praxis, Prager med. Wchnschr. **35**: 584, 1910. Viets, H. R., and Allen, A. W.: Unpublished observations quoted by Urmy, Ragle, Allen and Jones.[2e] Groen.[5]

The rapidity with which the polyneuritis of beriberi advances varies markedly in individual cases. There is evidence that in the total absence of the antineuritic vitamin in the diet about twenty days may be required for the appearance of the first symptoms.[2h] Should no treatment be instituted, the end stages may be reached within a matter of weeks. However, most patients suffer from a partial and irregular deficiency of the vitamin. Months may accordingly elapse before marked symptoms appear. With temporary increases in the amount of the vitamin ingested, remissions occur; these are followed by exacerbations as the diet again becomes inadequate. Some individuals may continue for long periods in a relatively stationary condition while partaking of diets containing an amount of vitamin B which is insufficient for recovery but enough to prevent further progression. Severely acute cases probably represent acute exacerbations precipitated perhaps by infections or excessive physical activity in persons who have had unrecognized mild symptoms of the disease for some time.[9]

It is not generally difficult to differentiate the clinical picture of vitamin B_1 deficiency from other forms of polyneuritis. In lead poisoning, as a rule, only the motor nerves and the anterior horn cells of the spinal cord are affected. Involvement of sensation is minimal and pain is rare. The upper extremity is more often affected. First there is a weakness of the hand, and this is followed by wrist drop within a few days. Foot drop occurs later. A history of colic, the appearance of a characteristic line on the gingival margins and basic stippling of the erythrocytes further aid in establishing the diagnosis. Polyneuritides due to the toxic action of the other heavy metals closely simulate the form due to lead.

Polyneuritis due to the toxic action of triorthocresyl phosphate, an occasional contaminant of jamaica ginger, and apiol and other abortifacients, like that due to poisoning by the heavy metals, is essentially motor in type. The onset is usually abrupt, with complete development of paralysis within a few days. Cresyl phosphate usually first affects the nerves of the legs, however. Many patients present foot drop alone. More advanced cases exhibit involvement of the hands and arms. Pain in the calves, without much tenderness, is a common but transient symptom at the onset. Disturbances of sensation are minimal.

Infectious polyneuritis (polyneuronitis, polyneuritis with facial diplegia) affects the proximal rather than the peripheral portions of the limbs. Involvement of the shoulder girdle and the upper thighs together with the cranial nerves, especially the seventh, is characteristic. The spread of weakness is toward the periphery rather than from it. Generally the protein content of the cerebrospinal fluid is increased, whereas in polyneuritic beriberi changes, if any, are slight.

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[9] Vedder, E. B.: Beriberi, in Tice: Practice of Medicine, Hagerstown, Md., W. F. Prior Company, 1929, vol. IX.

Diphtheria produces an ascending perineural lymphogenous intoxication commencing at the site of the infection. Faucial diphtheria thus first results in palatal paralysis, laryngeal diphtheria in laryngeal paralysis, only later spreading to contiguous centers in the nervous system. When the lesion is on the buttocks, as in diphtheritic saddle sores, the polyneuritis commences with involvement of the sacral nerves and then spreads up the cord.

Arsenical polyneuritis most closely simulates beriberi. However, this condition usually occurs after acute poisoning rather than from chronic intoxication, and hence the history is usually significant. Sensory changes are frequently more marked than motor changes. Burning, pain and paresthesias are particularly pronounced.

CARDIOVASCULAR MANIFESTATIONS OF VITAMIN B_1 DEFICIENCY

The cardiovascular manifestations of beriberi most commonly encountered are dyspnea and palpitation on exertion, tachycardia and edema.[10] The heart is generally enlarged both to the right and to the left. Systolic murmurs are common. Basilar pulmonary rales are frequent. The arterial blood pressure is usually normal or low, frequently with an increased pulse pressure. On palpation a bounding quality is noted in the larger arteries and "pistol shot" sounds may be heard on auscultation. The venous pressure is generally increased but may be normal.[11] The skin is usually warm and of normal color.[11] Cyanosis is rare; edema may be mild and only in dependent parts, or diffuse and extreme. Electrocardiograms generally show alterations, chiefly in the T waves.[12] Circulatory failure may be predominantly right sided or left sided. Sudden circulatory collapse (shock) has been observed, as well as syncope due to hypersensitivity of the carotid sinus.[11]

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[10] Aalsmeer, W. C., and Wenkebach, K. F.: Herz und Kreislauf bei der Beriberikrankheit, Wien. Arch. f. inn. Med. **16**: 193 (Jan.) 1929. Miura, K.: Beriberi oder Kakke, Ergebn. d. inn. Med. u. Kinderh. **4**: 280, 1909. Shimazono, J.: B-avitaminosis und Beriberi, ibid. **39**: 1, 1931. Keefer.[4] Weiss and Wilkins, footnotes 11 and 12.

[11] Weiss, Soma, and Wilkins, R. W.: The Nature of the Cardiovascular Disturbances in Vitamin Deficiency States, Tr. A. Am. Physicians **51**: 341, 1936.

It is thus apparent that the cardiovascular manifestations of vitamin B_1 deficiency do not, at least in the present state of our knowledge, comprise a rigid and easily recognized clinical syndrome. Furthermore, not infrequently rheumatic, arteriosclerotic or syphilitic heart disease may have superimposed injury due to vitamin B_1 deficiency.

However, there are technical measurements of the circulation which may prove to be of significant value in the differentiation of beriberi from other types of cardiovascular disorders. The circulatory minute volume and circulation time are both increased[13] in the cardiovascular complications of beriberi whereas other causes of congestive failure, except hyperthyroidism, result in conspicuous slowing of the circulation. The important points in establishing the diagnosis of cardiovascular disease dependent on deficiency of vitamin B_1 are, in addition to those points already presented, (1) the presence of other manifestations of vitamin B_1 deficiency, such as polyneuritis, or of deficiencies of other portions of the B complex, such as glossitis and pellagrous skin changes. Indeed it is rare to observe "beriberi heart" without at least minimal signs of polyneuritis; (2) the history of dietary inadequacy or of conditioning factors which lead to vitamin B_1 deficiency in spite of an apparently normal diet; (3) the disappearance of signs and symptoms following adequate B_1 therapy.

OTHER MANIFESTATIONS OF VITAMIN B_1 DEFICIENCY

A deficiency of vitamin B_1 leads to gastro-intestinal symptoms, chiefly anorexia and nausea. On the administration of vitamin B_1 these promptly disappear. There is, however, no method of differentiating a priori which clinical cases of anorexia and nausea are due to B_1 deficiency unless associated signs of B_1 deficiency are present. Even then such symptoms may be due not to a nutritional deficiency but to some associated condition such as alcoholic gastritis. There is, therefore, no justification for the indiscriminate treatment of anorexia with vitamin B_1. Similarly, although retardation of growth is found in B_1 deficiency, in the case of most underdeveloped children other causes are to be sought. Glossitis, achlorhydria, anemia and diarrhea are not infrequently seen in patients with vitamin B_1 deficiency. The evidence now at hand suggests, however, that these phenomena are manifestations of a deficiency of some portion of the vitamin B complex other than B_1.

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[12] (*a*) Weiss, Soma, and Wilkins, R. W.: The Nature of the Cardiovascular Disturbances in Nutritional Deficiency States (Beriberi), Ann. Int. Med. **11**: 104 (July) 1937; (*b*) footnote 11.

[13] Inawashiro, R., and Havasaka, E.: Studies on the Effect of Muscular Exercise in Beriberi, Tohoku J. Exper. Med. **12**: 1 (Dec. 30) 1928. Weiss and Wilkins footnotes 11 and 12.

A decade ago there was a failure on the part of many clinicians to recognize that vitamin B_1 deficiency was responsible for many cases of polyneuritis and cardiovascular dysfunction. Today the pendulum may be swinging too far in the opposite direction. There are instances in which lead poisoning, mediastinal tumors, cardiovascular syphilis and other conditions are being diagnosed as beriberi and treated as such to the exclusion of appropriate therapeutic measures. When doubt exists as to diagnosis, no harm will be done by administering vitamin B_1, but full investigation of the individual case must be carried out.

VITAMIN B_1 THERAPY

Vitamin B_1 may be employed in pure crystalline form or substances rich in vitamin B_1 content may be administered. For the definitely diagnosed case of beriberi, treatment may well be initiated by the intramuscular or intravenous injection of from 20 to 50 mg. of crystalline vitamin B_1 daily. This amount has appeared to be sufficient in all cases thus far observed and probably represents an excess over the actual requirement. However, overdosage entails no harm. As much as 100 mg. has been administered intravenously[12a] in one dose without ill effects of any kind. After a fortnight oral therapy may be employed in the same dosage or, if injections are continued, the dose is decreased to 10 mg. daily until the patient is completely relieved of all symptoms.

Brewers' yeast, plain or autolyzed, serves as a convenient means of administering not only vitamin B_1 but other portions of the B complex. Thirty grams of a powdered brewers' yeast of good potency administered three times daily will generally suffice for the treatment of the less seriously ill patients with vitamin B_1 deficiency, particularly if there is no reason to suspect the presence of gastro-intestinal disturbances which might interfere with the absorption of orally administered products. Since fatal vasomotor collapse can appear without warning in untreated patients with beriberi heart, it is of utmost importance that the administration of vitamin B_1 be commenced promptly.

It has often been pointed out that deficiency disease in man, unlike that experimentally produced in animals, is seldom limited to a single factor. A diet deficient in vitamin B_1 may possibly be low in other portions of the vitamin B complex as well as in vitamins A and C and in iron and other minerals. The frequent occurrence of glossitis, anemia and pellagra in association with beriberi suggests that many patients without such manifest signs nevertheless suffer from partial deficiency of other dietary factors. It is accordingly wise to provide that a patient with beriberi receive, in addition to proper amounts of vitamin B_1, an adequate amount of vitamins A and C and iron, as well as food rich in other portions of the vitamin B complex. The dilute liver extracts, suitable for intramuscular injection, given in dosage of from 10 to 20 cc. or more daily, have been particularly valuable in controlling glossitis and skin manifestations of the pellagrous type.

The results of vitamin B_1 therapy in both the polyneuritic and the cardiovascular manifestations of beriberi are most gratifying. When the polyneuritis is acute and not far advanced, one may observe complete remission of signs and symptoms in a matter of weeks. When, however, the nerve degeneration has been progressive over a long period of time and has reached a severe stage, a correspondingly long period will be required before complete recovery occurs. Peripheral nerves are capable of regeneration as long as the cell body in the spinal cord or posterior ganglion remains viable. Since this regeneration commences at the cell body and progresses distally at a rate of approximately 1 mm. a day, it is apparent that months will be required for the complete repair of advanced degeneration. Therefore, treatment must continue unremittingly until this occurs. Regeneration of completely degenerated cells and axis cylinders within the central nervous system does not take place. Accordingly, complete recovery of functions lost as a result of cerebral or posterior column cord degeneration cannot be expected, even though marked improvement in these functions not infrequently occurs.

The response of the cardiovascular system to adequate vitamin B_1 therapy is often dramatic. Within a few days the pulse rate is slowed, diuresis occurs and edema lessens. Within a fortnight the patient may lose 20 pounds (9 Kg.) or more of edema fluid, the electrocardiogram may return to normal, the heart may become noticeably smaller and the vital capacity of the lungs may increase markedly. In deficiency of long duration anatomic changes may occur in the heart muscle,[12a] so that five weeks or more may be required for the remission of all signs and symptoms. Repair of neural lesions in most instances occurs at an even slower rate than that of cardiovascular disturbances.

It should be emphasized that, striking as the results of vitamin B_1 therapy are in the cardiovascular disturbances arising from a deficiency of vitamin B_1, no effects whatever are to be expected in other types of heart disease, with or without edema, in the edema of pregnancy toxemias,[14] nephrosis and the like.

Other papers in this series bring out the limitations of our knowledge concerning the human requirements for vitamin B_1 and the vitamin B_1 content of foods. More definite data will be available in the future as the efforts of research workers in the laboratory and in the clinic continue unabated. The reports reviewed in the present paper indicate, within the limitations of the technic heretofore available, that vitamin B_1 deficiency is probably more commonly found in the United States than has been supposed. This condition is observed chiefly in association with diseases or bodily states that condition a deficient supply of vitamin B_1. The encouraging feature of clinical reports is that the condition is readily amenable to therapy.

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[14] Strauss, M. B.: Observations on the Etiology of the Toxemias of Pregnancy: III. The Lack of Influence of Vitamin B (B_1) on Water Retention in the Toxemias of Pregnancy, Am. J. M. Sc. **195:** 516 (April) 1938.

CHAPTER XI

VITAMIN B_1

METHODS OF ASSAY AND FOOD SOURCES

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The importance of vitamin B_1 in physiology and pathology has emphasized markedly the need of devising accurate methods for the quantitative determination of this substance in foods. Until recently the chemical identity of vitamin B_1 was unknown, rendering it impossible to develop chemical methods of analysis, and accordingly recourse was had to biologic methods of assay using rats and pigeons as test animals. Much information regarding the vitamin B_1 content of foods has been gained in this way, especially by the method in which the growth of rats was used as the measure of potency. This paper presents the technical aspects of various methods of bio-assay for vitamin B_1 from the point of view of quantitative interpretation of the results obtained and discusses briefly the chemical methods that are being developed. It concludes with an evaluation of foods as sources of the vitamin.

BIOLOGIC METHODS WITH PIGEONS

The first methods devised for the assay of vitamin B_1 were based on the prevention or cure of polyneuritis in pigeons.[1] There was also developed the weight maintenance method,[2] which involved the determination of the minimum amount of a substance required to maintain the weight of a standard bird on a polished rice diet. Because of many experimental difficulties, these methods are now used by only a limited number of investigators. The preventive method lacked specificity and the results were far too variable to admit of satisfactory quantitative analysis. The weight maintenance method raised the question of whether the antineuritic factor (vitamin B_1) and the growth factor were identical.[3] Although the curative method has been used more extensively than the others, it has not proved to be more than fairly satisfactory owing to several major difficulties, such as irregularity of appearance of polyneuritis, the development of multiple deficiencies because of the inadequacy of the polished rice diet, development of nonspecific symptoms due to inanition, temporary and spontaneous remission of symptoms, failure of cures and variability in the time of response in different birds. In this connection it is interesting to note that the prevention of polyneuritis in young chicks was recently suggested as a means of assaying for vitamin B_1.[4]

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[1] Emmett, A. D., and Peacock, G. E.: Adequacy of Pigeons and Rats for Vitamin B Studies, J. Biol. Chem. **63**: xxiii (Feb.) 1925. Kinnersly, O'Brien and Peters.[6] Coward, Burn, Ling and Morgan.[7]

[2] Seidell, Atherton: A Physiological Test for the Activity of Vitamin Preparations, Pub. Health Rep. **37**: 1519 (June 23) 1922. Plimmer, R. H. A.; Raymond, W. H., and Lowndes, John: Comparative Vitamin B_1 Values of Foodstuffs: Fruits and Vegetables, Biochem. J. **25**: 1788, 1931. Block, R. J.; Cowgill, G. R., and Klotz, B. H.: The Antineuritic Vitamin: I. The Method of Assay, Concentration of the Vitamin with Silver Under Various Conditions, and Its Solubility in Certain Organic Solvents, J. Biol. Chem. **94**: 765 (Jan.) 1932.

The methods referred to are described in the literature in more or less final form and it seems unnecessary to present them in detail here. However, certain results obtained with the pigeon curative technic, more especially those concerned with the derivation of quantitative values, are or may be paralleled in the rat curative method which is now under investigation by the Committee of Revision of the U. S. Pharmacopeia. It seems advisable, therefore, to review briefly the results obtained with the pigeon curative technic on the basis of their experience value.

In the procedure with the pigeon curative technic there seem to be several items directly influencing the results obtained. The magnitude of the response of the birds to test doses of vitamin B_1, for instance, is conditioned not only by the quantity of vitamin B_1 administered but also by such factors as the nature of the basal diet used, the duration of the initial depletion period and the duration of vitamin B_1 administration.[5] The method of administration of crystalline vitamin B_1 also affects the results in that the response to doses administered by injection is greater than the response to doses of the same size given orally.[6]

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[3] Smith, M. I.: A New Method of Evaluating the Potency of Antineuritic Concentrates, Pub. Health Rep. **45:** 116 (Jan. 17) 1930.

[4] Arnold, Aaron, and Elvehjem, C. A.: Studies on the Vitamin B_1 Requirements of Growing Chicks, J. Nutrition **15:** 403 (April) 1937.

[5] Carter, C. W., and O'Brien, J. R.: Maintenance Nutrition in the Pigeon: The Effect of Vitamin B_1, Biochem. J. **31:** 2264 (Dec.) 1937. Waterman, R. E., and Ammerman, Marion: Studies of Crystalline Vitamin B: VI. The Effect of Graduated Doses on Pigeons, J. Nutrition **10:** 161 (Aug.) 1935.

Several methods have been used or suggested for expressing the results of the assays quantitatively. In the very early tests, attempts were made to assess values by determining the minimum quantity (minimum curative dose) of the substance required to cure induced symptoms of polyneuritis. This procedure did not prove to be satisfactory because in practice it was not possible to define "minimum curative dose." [7] It soon became apparent also that the responses of different birds to like amounts of vitamin B_1 bearing material vary widely and that direct comparisons can be made only from successive tests with the same bird.

Further studies were then made to determine the relation between the size of the curative dose of vitamin B_1 administered and the duration of cure. Evidence was presented [8] to show that there is a direct quantitative relation between the dose and the duration of cure, i. e., the number of days of protection after cure before the onset of the next attack. In the application of this finding to the comparison of results with different substances, an attempt was made to reduce them to a comparable basis by expressing the relative potencies in terms of day doses per gram of material.[9] Values obtained from results with different dose levels of a given substance showed wide differences, however, and it was later concluded that this procedure is not a valid one.[6] The reason for this becomes clear when it is recognized that the relation of the duration of cure to weight of material given is not represented by a straight line.[7] It has been shown, however, that it is possible to derive a curve of reference representing the relation of duration of cure to quantity of vitamin B_1 ingested that is valid for use in interpreting results obtained by the curative method.[7]

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[6] Kinnersley, H. W.; O'Brien, J. R., and Peters, R. A.: Crystalline Vitamin B_1, *Biochem. J.* **29**: 701 (March) 1935.

[7] Coward, K. H.; Burn, J. H.; Ling, H. W., and Morgan, B. G. E.: The Determination of the Antineuritic Vitamin, Biochem. J. **27**: 1719 (No. 5) 1933.

[8] Kinnersley, H. W.; Peters, R. A., and Reader, Vera: Antineuritic Yeast Concentrates: III. The Curative Pigeon Test: A Critique, Biochem. J. **22**: 276 (No. 1) 1928.

[9] Kinnersley, H. W.; Peters, R. A., and Reader, Vera: A Quantitative Comparison of the Curative Activity of Torulin (Vitamin B_1) upon the Adult Pigeon and the Adult White Rat, Biochem. J. **24**: 1820 (No. 6) 1930.

Other estimates from data obtained with the pigeon curative method have been made on the basis of percentage of birds cured[7] and the degree of accuracy in these values was shown to be as great as in those computed on the basis of duration of cure.

The chief points to consider in judging the worth of a biologic method are its specificity, its precision and its sensitivity. In the curative method, precision is contingent on the determination of the length of the curative period, which in turn depends on the determination of the time of onset of the symptoms of polyneuritis. Even if the symptoms indicating the onset of polyneuritis could be exactly described, frequent periodic observation during the entire twenty-four hours of a recorded day would be necessary to ascertain the exact time of onset. In most laboratories such complete observations are not possible and the recorded time of onset of symptoms at the beginning and at the end of the curative period may be considerably in error. Thus the value given as the duration of the cure may involve an error in recorded time, as well as errors arising from the use of birds given the curative dose at different stages of development of polyneuritis.

The sensitivity of the test is also not so great as might be hoped for. A tenfold increase in vitamin B_1 intake apparently extends the curative period from the minimum of two or three days to only six or seven days. It seems justifiable to say, therefore, that the method is seriously lacking both in preciseness and in sensitivity.

Catatorulin Effect.—During the course of studies in which pigeons were used to determine the potency of vitamin B_1 concentrates, it was observed that the oxygen uptake of brain tissue from avitaminous pigeons increased on the addition of concentrates of vitamin B_1 in lactate solution.[10] This was interpreted to be in the nature of a catalytic effect and the active factor was designated catatorulin although there was strong evidence that it was identical with vitamin B_1. A technic was devised whereby the effect of the vitamin B_1 containing substance on the oxygen uptake of excised pigeon's brain was used as an index of the quantity of vitamin B_1 present.[11] The curve relating the dose of vitamin B_1 (micrograms to 3 cc. of solution) to oxygen uptake (milligrams per gram hourly), although not a straight line, was a smooth curve similar to the one relating vitamin B_1 intake to growth in rats. The catatorulin test has been suggested as a most promising one for comparing the potencies of vitamin B_1 containing substances, although further study is required to perfect the method.[6]

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[10] Passmore, Reginald; Peters, R. A., and Sinclair, H. M.: On Catatorulin: A New Method of Comparing the Oxidative Factor in Vitamin B_1 Concentrates, Biochem. J. **27**: 842, 1933.

BIOLOGIC METHODS WITH RATS

The Growth Test.—The use of growth in the rat as a means of detecting the presence of vitamin B_1 in substances was suggested as early as 1918. The method developed by Sherman and Spohn[12] and more especially the basal diet worked out by them was generally used up to the time when it was shown that for normal growth the rat requires in addition to vitamin B_1 a heat stable fraction remaining in autoclaved yeast. The Sherman and Spohn basal diet was then modified by Chase[13] to include sufficient autoclaved yeast so that vitamin B_1 was the first if not the only growth limiting factor.

The use of a preliminary depletion period was also suggested at this time as a precautionary measure even though it is generally agreed that vitamin B_1 is not stored in the body to any great extent and that animals given a vitamin B_1 free diet show only slight residual growth.

The procedure of the rat growth technic,[14] like that of any other method, is carried out more or less according to the tenets of the investigator who is using it. For the purpose of this discussion, however, it does not seem necessary to consider in detail the various conclusions and recommendations that have been made except so far as they have a direct bearing on the uniformity and reliability of the results obtained.

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[11] Peters, R. A.; Rydin, Hakan, and Thompson, R. H. S.: Brain Respiration, a Chain of Reactions, as Revealed by Experiments upon the Catatorulin Effect, Biochem. J. **29**: 53 (No. 1) 1935. Passmore, Peters and Sinclair.[10]

[12] Sherman, H. C., and Spohn, Adelaide: A Critical Investigatio nd an Application of the Rat Growth Method for the Study of Vitami B, J. Am. Chem. Soc. **45**: 2719 (Nov.) 1923.

[13] Chase, E. F., and Sherman, H. C.: A Quantitative Study of the Determination of the Antineuritic Vitamin B, J. Am. Chem. Soc. **53**: 3506 (Sept.) 1931.

[14] British Pharmacopeia, Biological Assay of Antineuritic Vitamin (Vitamin B_1), 1932, p. 91. Chase and Sherman.[13] Coward, K. H.: The Biological Standardization of the Vitamins, London, Baillière, Tindall and Cox, 1938.

Essentially, the procedure consists in the keeping of healthy young rats of known lineage and within a given age and weight range on a diet devoid of vitamin B_1 but adequate in all other factors needed for growth. They are maintained on this diet without supplement either for a specified length of time considered sufficient for depletion or until there are evidences that their stores of vitamin B_1 have been depleted. The animals are then given daily (or six times a week) weighed quantities of the material under investigation during a test period of specified length. The gain in weight during this period is used as a measure of the quantity of vitamin B_1 present.

The reliability of the results from the rat growth method depends in large part on the selection and grouping of the experimental animals. The quantity of the standard material fed should be that which gives a growth response at or near the critical level, this being the gain in weight from which comparisons are to be made. In the Sherman-Chase method, for instance, the point corresponding to an average gain of 3 Gm. a week is used. This point is located in the steepest part of the curve relating gain in weight to dose of vitamin B_1 but far enough up on the curve so that all the experimental animals may be expected to survive. It is often the practice to administer the test material at three or more dose levels and the Standard of Reference material at one or more dose levels. The quantities of test material are so selected that one induces a growth response at or near the critical level, one above this and one below.

The animals to be given the several doses must be as nearly alike as possible. On identical quantities of vitamin B_1 the growth response of males is usually greater than that of females.[15] Animals of different strain, or even litters of the same strain, may show differences in growth response for given quantities of vitamin B_1.[16] For these reasons it is also recommended that the series of graded test doses and the dose of the standard be allotted to littermate animals of the same sex of about the same weight. Additional litters of animals are then used until the number of individuals on each dose level is sufficient to give an average result having an acceptable degree of reliability.

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[15] Sherman, H. C., and MacArthur, E. H.: A Quantitative Study of the Determination of Vitamin B, J. Biol. Chem. 74:107 (July) 1927. Coward, Burn, Ling and Morgan.[7] Waterman and Ammerman.[20]

In the original Sherman and Spohn technic a test period of eight weeks was used. It was felt later, however, that results from a shorter period might more truly represent an uncomplicated vitamin B_1 effect, free from influence of other unknown growth essentials that might not have been supplied in the basal diet. Accordingly, a four weeks period was suggested.[17] Results from a test period of two weeks have been reported to be very nearly as good as those from one of three weeks' duration.[18] With a low rate of gain, results have been obtained on the basis of a ten day period,[19] although a somewhat longer period than this would seem to be generally more satisfactory.

At the completion of the test the gains in weight for the individual animals in each group are determined and the average gains in weight for the different groups are calculated. If the test material contained vitamin B_1 the gains in weight will be graded to the dose. With crystalline vitamin B_1, for instance, growth has been shown to increase with doses as high as 160 micrograms per rat daily.[20]

From the results obtained a curve (curve of response or dose-effect curve) is drawn, relating gain in weight to quantity of material given. The smoothness of this curve will serve as an indication of the reliability of the test. The quantity of test material estimated to give the same effect, i. e. to have the same potency as the quantity of standard fed, is then derived from the curve of response, which is drawn to a suitable scale so that the degree of precision implied by the value derived is in accord with the values used in constructing the curve.

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[16] Light, R. F., and Cracas, L. J.: Vitamin B_1 Requirements of Different Strains of White Rats, Science **87**: 90 (Jan. 28) 1938.

[17] Sherman, H. C., and Smith, S. L.: The Vitamins, New York, Chemical Catalog Company, 1931, p. 103.

[18] Coward, K. H.: The Influence of the Length of the Test Period on the Accuracy Obtainable in a Vitamin B_1 Test, Biochem. J. **30**: 2012 (Nov.) 1936. Sherwood, F. W., and Halverson, J. O.: A Critical Evaluation of the Rat-Growth Method for Determining Vitamin B and Its Content in Meals from Certain Oily Foods, J. Agric. Res. **56**: 927 (June 15) 1938.

[19] Schlutz, F. W., and Knott, E. M.: The Use of a Ten-Day Period for the Assay of Vitamin B by Rat Growth Technic, J. Nutrition **12**: 583 (Dec.) 1936.

[20] Waterman, R. E., and Ammerman, Marion: Studies of Crystalline Vitamin B: V. The Effect of Graduated Doses on Growing Rats, J. Nutrition **10**: 35 (July 10) 1935.

The degree of accuracy of vitamin B_1 estimated by the rat growth method has been studied statistically and is reported to be high for a method of this type.[18] The sensitivity also seems to be high, since it is possible to differentiate doses of crystalline vitamin B_1 differing in potency by only one microgram, although a dose of three micrograms must be administered before an appreciable growth response is obtained.[21]

Refection and Coprophagy.—One of the more serious difficulties involved in the vitamin B_1 assay is that of eliminating the extraneous source of vitamin B_1 to which the rat may have access as a result of "refection." [22] There is ample evidence for believing that vitamin B_1 may be formed in the cecum probably as the result of bacterial action on the food residues there.[23] Refection appears to be dependent on the starch component of the diet. Raw potato starch, for instance, has a high refective power, while rice, wheat and rye starches are reported to have little or none.[24] Cooking the starch seems to destroy its refective property.[25] The vitamin B_1 due to refection is not absorbed from the lower intestinal tract, but the rat may obtain benefit of it by the simple expedient of consuming his feces. The use of cages with large mesh screen bottoms tends to lessen this tendency but does not necessarily eliminate it altogether. One consoling feature with regard to refection is that it does not occur in all cases and a watchful observer can generally identify refected animals by the bulky character of the feces and a growth response not in accord with the known intake of vitamin B_1.

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[21] *Unpublished data of Bureau of Home Economics, U. S. Department of Agriculture.*

[22] Steenbock, Harry; Sell, M. T., and Nelson, E. M.: Vitamin B: I. A Modified Technic in the Use of the Rat for Determination of Vitamin B. J. Biol. Chem. **55:** 399 (March) 1923. Dutcher, R. A., and Francis, Emma: Vitamin Studies: X. Feeding Technic in Vitamin Studies. Proc. Soc. Exper. Biol. & Med. **21:** 189 (Jan.) 1924. Fridericia, L. S.; Freudenthal, P.: Gudjonnsson, S.; Johansen, G., and Schoubye, N.: Refection, A Transmissible Change in the Intestinal Content, Enabling Rats to Grow and Thrive Without Vitamin B in the Food, J. Hyg. **27:** 70 (Nov.) 1927.

[23] Guerrant, N. B.; Dutcher, R. A., and Tomey, L. F.: The Effect of the Type of Carbohydrate on the Synthesis of the B Vitamins in the Digestive Tract of the Rat, J. Biol. Chem. **110:** 233 (June) 1935. Griffith, W. H.: Studies on Growth: III. B and G Avitaminosis in Cecectomized Rats, J. Nutrition **10:** 667 (Dec.) 1935. Abdel-Salaam, A., and Leong, P. C.: Synthesis of Vitamin B_1 by Intestinal Bacteria of the Rat, Biochem. J. **32:** 958 (June) 1938.

[24] Kon, S. K.: Potato Starch and Refection, J. Hyg. **31:** 543 (Oct.) 1931. Bliss, Sidney, with the technical assistance of Green, Fred: Refection in the Rat: With an Appendix on Methods of Preparing Basic Materials for Deficient Diets, J. Nutrition **11:** 1 (Jan.) 1936.

[25] Roscoe, M. H.: Spontaneous Cures in Rats Reared upon a Diet Devoid of Vitamin B and Antineuritic Vitamin, J. Hyg. **27:** 103 (Nov.) 1927.

The Curative Technic.—Polyneuritis in rats was reported as early as 1917 and in the following year a method for vitamin B_1 determination was described in which rats were kept on the vitamin B_1 free diet until they showed declining weight or symptoms of paralysis or both. Hofmeister [26] in 1922 was the first, however, to report the consistent appearance of polyneuritis in large numbers of rats. Several years later a comparison of results by the pigeon test and the rat curative test led to the conclusion that the same factor was measured with rats as with pigeons.[9] About the same time Sandels,[27] using a basal diet containing adequate amounts of the heat stable components, reported that polyneuritis occurred in rats only when small quantities of vitamin B_1 were present in the basal diet. This point has since been corroborated by other investigators.[28]

The first plausible plan for vitamin B_1 determination based on the curative technic with rats was that described by Smith.[29] Rats from 50 to 70 Gm. in weight and from 30 to 40 days of age developed symptoms of polyneuritis in from fifty to eighty days. Vitamin B_1 administered orally or by injection produced improvement in from three to five hours and a definitely curative effect in from eighteen to twenty-four hours. When the curative effect of a given dose had worn off the symptoms of polyneuritis recurred and were again alleviated by a dose of the vitamin. It was suggested that the same animal might be used many times with little variation in its response to doses of equal potency. Although Smith found the curative effect proportional to the dose, the relationship was not considered quantitative and he made his comparisons, therefore, on the basis of the minimum curative dose. A cure lasting three days was considered the shortest remission period a minimum effective dose would bring about. The method was checked with a large number of substances and was considered to be entirely specific.

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[26] Hofmeister, Franz: Studien über qualitative Unterernährung: II. Mitteilung der experimentelle Nachweis des Antineuritins, Biochem. Zschr. **129:** 477, 1922.

[27] Sandels, M. R.: Experimental Nutritional Polyneuritis in the Rat, J. Nutrition **2:** 409 (March) 1930.

[28] Sebrell, W. H., and Elvove, E.: Observations on the Assay of the Antineuritic Vitamin: Some of the Factors Involved in the Use of the Rat Method, Pub. Health Rep. **46:** 917 (April 17) 1931. Waterman and Ammerman.[20]

[29] Smith, M. I.: A New Method of Evaluating the Potency of Antineuritic Concentrates, Pub. Health Rep. **45:** 116 (Jan. 17) 1930.

One particular advantage claimed for the foregoing method is the possibility of making comparative tests with the same animal, thus eliminating some of the errors due to differences in behavior of different test animals, a defect inherent in the rat-growth method. Several investigators working with the curative method have, however, reported considerable variation in the occurrence of polyneuritis and it is suggested that complete familiarity with the symptoms is essential before assay work is undertaken.[30] In this connection, criteria for judging the presence of polyneuritis have been described in detail and attention has been called to the fact that treatment of subsequent onsets may not always produce exactly the same result. The effect of such variation is reduced by the use of a sufficiently large number of animals.

To obtain greater uniformity in the occurrence of polyneuritis, the administration of known quantities of vitamin B_1 to each test animal has been recommended. Ammerman and Waterman,[31] for instance, suggest the daily injection of 0.5 microgram of crystalline vitamin B_1. Dann[32] uses a diet containing 400 Gm. of brewers' yeast per hundred pounds, which she claims gives a higher incidence of polyneuritis than the diets used by Ammerman and Waterman or Smith. Recent work[33] seems to indicate that the fat content of the diet may be an important factor in vitamin B_1 tests in relation to the production of polyneuritis in rats. A low incidence of polyneuritis was found among rats on a high fat diet, which is in accord with other observations on the fat-sparing action of vitamin B_1.

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[30] Heyroth. F. F.: The Evaluation of Vitamin B_1 Concentrates by the Rat Cure Method. Bull. Basic Science Research **4**: 1, 1932.

[31] Ammerman, Marion. and Waterman, R. E.: Studies of Crystalline Vitamin B: IV. Injection Method of Assay, J. Nutrition **10**: 25 (July) 1935.

[32] Dann. Flemintine P.: Vitamin B Assay Using Rat Curative Method with Modified Diets and Oral Administration of Addenda, J. Nutrition **12**: 461 (Nov.) 1936.

[33] McHenry, E. W.: An Effect of Choline on the Vitamin B_1 Sparing Action of Fats, Biochem. J. **31**: 1616 (Sept.) 1937. Salmon, W. D., and Goodman, J. G.: Alleviation of Vitamin B Deficiency in the Rat by Certain Natural Fats and Synthetic Esters, J. Nutrition **13**: 477 (May) 1937.

As already stated, studies for the purpose of selecting a suitable method for the assay of vitamin B_1 are at present being carried out under the direction of the Committee of Revision of the Pharmacopeia of the United States of America. Only the rat curative technic is being considered because a previous survey had indicated this to be the preferred method of the four procedures to which consideration had been given. Of the several modifications of the rat curative technic under investigation it seems necessary, however, to consider only the one in use at present in the Vitamin Division of the Food and Drug Administration of the United States Department of Agriculture.[34] This procedure is based on the one used by Smith, differing from it primarily in the use of a more adequate diet consisting of sucrose 61.25 per cent, casein 18.0 per cent, salt mixture I 4.0 per cent, autoclaved yeast 4.0 per cent, autoclaved peanuts 10.0 per cent, purified liver extract 0.75 per cent, and cod liver oil 2.0 per cent.

Sources of all known components of the vitamin B complex, including vitamin B_4 and factor W of Elvehjem, are presumably present in this diet. Special purification of the casein and the use of sucrose in place of starch are also new features. Although a known source of the minimal quantity of vitamin B_1 considered to be essential for the production of polyneuritis is not included in this diet, 100 per cent occurrence of polyneuritis in rats weighing from 40 to 70 Gm. is claimed for it. Furthermore it has been demonstrated that, with this diet, polyneuritis can be produced and cured repeatedly in the same animal, the production of ten or more such periods in a single animal being quite usual.

The details of the test may be summarized briefly. The basal diet is given to litters of rats from 12 to 13 days of age, which are kept with their mothers in cages having raised screen bottoms. The young are weaned when they weigh between 40 and 50 Gm. and are weighed weekly until the twenty-fifth day. At the end of this period they are put in individual cages, weighed daily and observed for symptoms of polyneuritis, which are classified as slight, acute or severe. Only animals in the acute stage are used in an assay.

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[34] Kline, O. L.; Tolle, C. D., and Nelson, E. M.: Vitamin B_1 Assay by a Rat-Curative Procedure, J. A. Off. Agric. Chem. **21:** 305 (May) 1938.

When an animal is judged ready for the test, 2 international units of vitamin B_1 are administered and the weight and condition of the animal are again noted daily. The time elapsing between the administration of the curative dose and the recurrence of polyneuritic symptoms is designated the curative period. At the second occurrence of acute polyneuritis, a dose of the test material is given and the length of the curative period is recorded as before. In Kline's experience 2 international units of vitamin B_1 gives a curative period of from seven to eleven days with animals ranging in weight from 40 to 70 Gm. at the time the curative dose is administered.

Results obtained in the assay of crystalline vitamin B_1 by this method show that the duration of cure following administration of vitamin B_1 increases with increasing dosage. For a considerable range the relationship is linear, although at higher levels the curve flattens off as do other dose-effect curves showing the behavior of animals to graded doses of a given vitamin. In successive periods with the same animal the length of the curative period for a given dose of crystalline vitamin B_1 was found to be uniformly reproducible to a remarkable degree. Thus it was considered possible to use the length of the curative period as an accurate measure of the vitamin B_1 administered in a test dose. Errors due to variation of animals are eliminated by using the results of successive trials with the same animal to compare the potency of the test material with that of the standard.

In an assay performed in this way a given quantity of the test substance is considered to contain at least 2 international units of vitamin B_1 if it promotes in the experimental animals a total curative period (the sum of the days of the curative period of each of the animals) equal to or greater than the total curative period following the feeding of an amount of the international standard equivalent to 2 units. "If it is desired to determine the exact potency of an unknown [test material], it is preferable to feed it to a few animals at various levels to determine the approximate quantity which contains two international units of vitamin B_1. The assay should then include successive feedings of the standard and unknown [test material] on at least eight animals." It is the opinion of the sponsor of this method that it is the most accurate procedure for vitamin B_1 determination that has been proposed.

Several measures other than duration of cure have been used for the quantitative expression of results from the rat curative technic. One of the first was weight recovery time,[30] i. e. the time required for the rat to return to the weight it showed when the injection of vitamin B_1 was made. Another measure used is the number of day doses per gram of material.[9] This value is derived in the same way as in the method with pigeons and is therefore subject to the same limitations.[35] Reference has already been made to the minimum curative dose used in the method described by Smith.[29] Although this measure is more logical than the determination of day doses it involves the same difficulties associated with the determination of the minimum curative dose for pigeons.

The Bradycardia Method.—In 1929 a condition of heart block and bradycardia preceding appearance of symptoms of polyneuritis or head retraction was reported in pigeons fed polished rice. The following year a condition of bradycardia in rats was described[36] which did not seem to be due to lowered food intake but rather to the effects of lack of vitamin B complex, since the condition did not occur in controls on a restricted food intake. It was cured by administration of vitamin B_1. A special device for taking electrocardiograph records had been designed and the average normal heart rate for the rat was determined as from 500 to 530 per minute. Rats maintained on a vitamin B_1 free diet for about two weeks showed a gradual slowing of the heart rate, which finally reached a low level of 250-300 beats per minute. On the administration of vitamin B_1 [37] the rate returned to normal and remained so for several days, when it began to fall again. The degree of recovery was "related" to the quantity of vitamin B_1 available.

Following this a method for the quantitative determination of vitamin B_1 based on the remission of bradycardia in rats was suggested.[38] In this method young rats weighing about 40 Gm. are given a vitamin B free diet and after about three weeks, or when loss of weight begins, electrocardiograms are taken. As soon as a heart rate of 350 beats per minute is recorded, the test sample is given and "dosing" is repeated until there is an increase in the rate. Thereafter, readings are taken every twenty-four hours until the heart rate has fallen again to the level recorded at the beginning. Both the increase in rate and the duration of the increased rate were found to be proportional to the dose administered. Cures of four days' duration are considered most satisfactory. Tests are made at several levels with from four to six animals at each level and a dose-effect curve is plotted. Comparisons are made to determine the quantities of the international standard that give the same effect. An activated clay product, yeast, marmite and wheat germ, were assayed by the procedure described and also by the rat curative method, the rat growth method and the pigeon technic. The results by all four procedures agreed.[38]

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[35] Heyroth, F. F.: The Use of Various Rat Assay Methods in Comparing Crystalline Vitamin B_1 Preparations, Biochem. J. **30:** 645 (April) 1936.

[36] Drury, A. N.; Harris, L. J., and Maudsley, Cecil: Vitamin B Deficiency in the Rat: Bradycardia as a Distinctive Feature, Biochem. J. **24:** 1632 (No. 6) 1930.

[37] An autolyzed yeast preparation (Marmite) was used.

The advantages claimed for the bradycardia method are a short preparatory period, ease and rapidity in obtaining results, and economy in test animals since one animal may be used for several tests. The sensitivity is considered to be only slightly less than that of the growth method. The investigators admit that the chief drawback to the general use of this method is the expense of the electrocardiograph. It has been used by Baker and Wright [39] for the assay of about a hundred different foods. With 20 and 30 mg. of the International Standard of Reference, the method was considered accurate to within ± 20 per cent. Cures giving responses of about 450 beats per minute tended to be prolonged or irregular. The responses to the first test doses tended to be abnormal and these results, therefore, were discarded. The values reported by these investigators are in general somewhat higher than values given for similar products by other investigators.

The original proponents of this method have made a thorough study of it in a determination of the potency of crystalline vitamin B_1 preparations and have also used it extensively and apparently successfully in studies of the urinary excretion of vitamin B_1.[40] In the hands of others, however, it has not fared so well. Robertson and Doyle,[41] who attempted to use it in the assay of a crystalline vitamin B_1 preparation, observed considerable fluctuation in individual animals. A single tracing, for instance, showed a rate in one part that was forty beats per minute higher than the rate in another part, and two tracings from one rat showed differences of 100 beats per minute. Another rat on a curative dose of 6 micrograms showed rates varying from 326 to 480. R. S. Harris,[42] working with a carniotachometer designed for the purpose, has found discrepancies of even greater magnitude in normal and in vitamin B_1 deficient rats.

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[38] Birch, T. W., and Harris, L. J.: Bradycardia in the Vitamin B_1 Deficient Rat and Its Use in Vitamin B_1 Determinations, Biochem. J. **28**: 602 (No. 2) 1934.

[39] Baker, A. Z., and Wright, M. D.: The Vitamin B_1 Content of Foods, Biochem. J. **29**: 1802 (July) 1935.

Fermentation Test.—A fermentation test has been described for vitamin B_1 based on the powerful action exerted by the vitamin on the rate of alcoholic fermentation. The quantitative method involves measurement of the rate of gas evolution and with a suitable sugar-salt buffer mixture as little as one microgram of the vitamin may be detected.[42a]

Growth of Staphylococcus aureus.—The growth of Staphylococcus aureus has been shown to be proportional to the amount of vitamin B_1 in the culture medium. This is suggested as the basis for a method of measurement of the vitamin and is a suitable medium containing other substances such as nicotinic acid that are known to accelerate its growth has been described for use in making the test. The rate of growth due to

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40. Harris, L. J., and Leong, P. C.: Vitamins in Human Nutrition: The Excretion of Vitamin B_1 in Human Urine and Its Dependence on the Dietary Intake, Lancet **1:** 886 (April 18) 1936. Leong, P. C., and Harris, L. J.: Antineuritic Potency of Synthetic and Natural Crystalline Vitamin B_1 as Determined by the "Bradycardia" Method with a Statistical

Index of the Nutritional Level, Lancet **1:** 539 (March 5) 1938.

41. Robertson, E. C., and Doyle, M. E.: Difficulties in the Use of Bradycardia Method of Assaying Vitamin B_1, Proc. Soc. Exper. Biol. & Med. **37:** 139 (Oct.) 1937.

42. Harris, R. S.: Personal communication to the author, from the Massachusetts Institute of Technology, Cambridge, Mass.

42a. Schultz, A.; Atkin, L., and Frey, C. N.: A Fermentation Test for Vitamin B_1, J. Amer. Chem. Soc. **59:** 948 (May) 1937. Schultz, A. S.; Atkin, L., and Frey, C. N.: A Fermentation Test for Vitamin B II, J. Amer. Chem. Soc. **59:** 2457 (Nov.) 1937. Schultz, A. S.; Atkin, L., and Frey, C. N.: Influence of Nicotinic Acid on the Fermentation Method for Vitamin B_1 Determination, J. Amer. Chem. Soc. **60:** 1514 (June) 1938.

added vitamin B_1 is determined, after incubation, by turbidity measurements made with an Evelyn Electrophotometer. Growth increments are shown with as little as 0.00005 micrograms of vitamin B_1 per ml. and increases up to 0.001 micrograms per ml.[42b]

QUANTITATIVE INTERPRETATION OF RESULTS BY THE BIOLOGIC METHODS

The variety of assay procedures described naturally makes one query concerning the quantitative interpretation of results. Those obtained in the early studies in which pigeons were used were interpreted either directly in terms of the curative dose, as already explained, or in relation to the potency of some other substance such as yeast assigned an arbitrary value, for example 1, 10 or 100. These data are difficult, if not impossible, to reconcile with values obtained by other methods and at most have little more than qualitative significance. It is probably safe to say that many of the data on the occurrence of vitamin B_1 obtained by the pigeon technic prior to the time of adoption of the international standards have not been quantitatively evaluated. This may in part account for the dearth of tabulated vitamin B_1 values.

Two units other than the international unit have been defined for the interpretation of data by the rat growth method. The first was the maintenance unit, which was defined [43] as that amount of vitamin B_1 which, when fed as a daily allowance, results in net maintenance of a standard rat over an eight weeks period under the conditions described. Although this unit was never used extensively and there are few data reported in terms of it, Cowgill has referred to it in his discussion of equivalents and for this reason it seems advisable to mention it here.

The large majority of values for the vitamin B_1 content of foods as well as for other substances has been expressed in terms of the growth unit. This is defined [44] as that amount of vitamin B_1 which, when fed daily under experimental conditions as described, induces in a standard test animal an average gain of 3 Gm. a week during the test period. Values expressed in this unit have been derived from results obtained with the Sherman-Spohn diet as well as the Sherman-Chase basal diet. The Sherman-Spohn diet did not contain a supply of vitamin G sufficient for growth, and values obtained with this diet therefore may or may not indicate the full vitamin B_1 value of the substance tested, depending on whether the basal diet and the test substance supplied sufficient vitamin G for growth. Roscoe[45] used a somewhat higher rate of gain, from 10 to 12 Gm. a week, for the growth unit.

[42b] West, P. M., and Wilson, P. W.: Biological Determination of Vitamin B_1 (Thiamin) in Rhizobium Trifolii, Science **88**: 334 (Oct. 7) 1938.

[43] Sherman, H. C.: Chemistry of Food and Nutrition, ed. 3, New York, Macmillan Company, 1926, p. 406.

[44] Sherman, H. C.: Chemistry of Food and Nutrition, ed. 5, New York, Macmillan Company, 1937, p. 395.

The introduction of the international standards has given a more precise method of expressing vitamin values than was possible before these standards became available. There is evidence, however, that estimates made in terms of international units are not always as precise as they might have been. The derivation of values by the use of standards of reference by any method of analysis involves the determination of quantities of standard and of test material that give the same effect. The accuracy of the assay depends on the closeness of this determination. Many investigators use the method of trial tests to determine the quantity of test material equivalent to a given quantity of standard. With the wide variations inherent in results obtained by biologic methods a close match is obtained only if great care is taken in carrying out the assay; and, even when such results seem to have been obtained, the reliability is even more difficult to check. A perusal of published data indicates that in this procedure more attention should be given to the adoption of criteria for determining the limits within which the two effects may be considered to be identical. Other investigators may use a dose effect curve, as described under the growth method with rats, from which the quantity of test material equivalent to the quantity of standard used may be estimated if the results of the assay do not give it directly. Such curves may involve large errors of estimate, however, unless consideration is given to factors giving rise to variation in results with different test subjects.

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[45] Roscoe, M. H.: The Distribution of the Vitamin B Complex: I. Leafy Vegetables, Biochem. J. **24**: 1754 (No. 6) 1930.

The recently completed assay of the crystalline vitamin B_1 hydrochloride proposed for international standard to replace the adsorbate now in use indicates that vitamin B_1 values for substances assayed against the international standard adsorbate vary according to the method of assay used. Values obtained by the curative technics, for instance, were considerably higher than values by the prophylactic or growth method. It is suggested that in sick animals such as those used in the curative method there is incomplete elution of vitamin B_1 from the adsorbate in the alimentary canal. It has been shown[46] that rats seriously depleted of vitamin B_1 cannot fully utilize the vitamin B_1 present in preparations such as fullers' earth adsorbates. In the curative method, therefore, the response to the international standard adsorbate does not represent the full vitamin B_1 activity of the dose given and the vitamin B_1 potency of the test substance determined by this method is accordingly judged to be higher than it really is. In the growth method, on the other hand, the animals are healthy and seem to utilize fully the vitamin B_1 of the standard adsorbate. Vitamin B_1 values by the growth method are therefore probably more nearly true values although considerably lower than those obtained by the curative technics.

Whether this same relationship holds for natural products depends on the availability of vitamin B_1 in these products.

Since the adoption of the international standards, attempts have been made to derive a factor for converting vitamin B_1 values expressed in Sherman units to equivalent values in international units. Values from 1 to 4 Sherman units equivalent to 1 international unit have been suggested. As explained in an earlier paper of this series,[47] the growth units have no fixed value in terms of the vitamin for which they are defined and therefore conversion factors cannot be derived exactly.

Many food products present a difficulty in vitamin B_1 assays not experienced so generally in the case of other vitamins. Although vitamin B_1 occurs in a wide variety of foods, the quantity present in most cases is small. This means that the test doses used in the biologic assays must be rather large and it is often difficult, if not well nigh impossible, to get the animals to consume enough of the allotted portions to make the test reliable. Furthermore, it may not be possible to obtain responses that are divergent enough to indicate the dose effect relation essential to the derivation of a true estimate of the potency of the test material.

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[46] Keresztesy, J. C., and Sampson, W. L.: Utilization of Vitamin B_1 from Fullers' Earth Adsorbates, Proc. Soc. Exper. Biol. & Med. **36:** 686 (June) 1937.

[47] Munsell, Hazel E.: Vitamin A: Methods of Assay and Food Sources, J. A. M. A. **111:** 245 (July 16) 1938.

The foregoing discussion is intended to imply that vitamin B_1 values that have been reported, especially those of unusual magnitude, should not be accepted categorically apart from a consideration of the method of assay used and the exactitude with which the values were derived from the results obtained. When using tabulated values expressed in international units it is advisable to scrutinize them carefully to ascertain whether they were determined experimentally in international units or were derived by the use of a conversion factor from values determined in terms of some other unit.

CHEMICAL METHODS OF ANALYSIS

Although several chemical methods of analysis have been suggested for the determination of vitamin B_1, the thiochrome method is the only one that has been studied to any great extent. Early in 1935 Peters[48] reported that the purest preparations of vitamin B_1 available were converted by oxidation in aqueous solution into substances showing intense sky-blue fluorescence. Somewhat later, Barger, Bergel and Todd[49] succeeded in transforming vitamin B_1 into a pale yellow sulfur-containing compound by oxidizing it in alkaline solution with potassium ferricyanide. The compound formed showed an intense blue fluorescence in neutral or alkaline solution and had all the properties of the thiochrome described by Kuhn and his associates.[50] Jansen[51] studied the quantitative transformation of thiamin to thiochrome and concluded that the reaction took place at an optimal p_H of 10 and the maximum reaction in one or two minutes. Excess potassium ferricyanide seemed to destroy the thiochrome. The thiochrome was then extracted with isobutanol and the extract transferred to a Cohen fluorimeter. The test with the international standard was made by eluting the vitamin with a mixture of alkali and potassium ferricyanide. The procedure of alkali extraction was later adopted for removing the vitamin from other materials. Westenbrink and Goudsmit[52] applied the method of Jansen to the determination of vitamin B_1 in urine samples. The urine was diluted with three volumes of water and the reaction was carried out in an atmosphere of nitrogen. Errors due to interfering fluorescent substances were eliminated by "subtracting the fluorescence of a franconite eluate to which no potassium ferricyanide had been added from the fluorescence of some eluates which had been treated with different amounts of this oxidizing agent." Results by this technic agreed well with those obtained by Harris and Leong using the bradycardia method. Pyke[53] made a rather extensive study of the method as applied to the determination of vitamin B_1 in foods. The report covering this study gives a detailed description of the procedure, in which fluorescence was measured by a Cohen fluorimeter. Compensation for variation in intensity of the fluorimeter was obtained by reading the deflexion given by 10 cc. of solution containing 0.81 mg. of quinine sulfate in 100 cc. of tenth normal sulfuric acid. Readings from solutions of thiochrome were calculated back on the basis of 100 for this deflection. With the pure vitamin the fluorimeter readings and the concentrates of vitamin showed a straight line relationship. In the procedure as finally adopted the fluorescence given by a solution to which no potassium ferricyanide had been added was subtracted from the fluorescence of the treated solution to get that due to thiochrome.

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[48] Peters, R. A.: Vitamin B_1 and Blue Fluorescent Compounds, Nature **135**: 107 (Jan. 19) 1935.

[49] Barger, G.; Bergel, F., and Todd, A. R.: A Crystalline Fluorescent Dehydrogenation Product from Vitamin B_1, Nature **136**: 259 (Aug. 17) 1935.

[50] Kuhn, Richard; Wagner-Jauregg, Theodor; Van Klaveren, F. W., and Vetter, Hellmuth: Ueber einen gelben, schroefelhaltigen Farbstoff aus Hefe, Ztschr. f. physiol. Chem. **234**: 196, 1935.

[51] Jansen, B. C. P.: A Chemical Determination of Aneurin (Vitamin B_1) by the Thiochrome Reaction, Rec. trav. chim. **55**: 1046, 1936.

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[52] Goudsmit, J., and Westenbrink, H. G. K.: Determination of Aneurin (Vitamin B_1) in Urine by the Thiochrome Method, Nature **139:** 1108 (June 26) 1937. Westenbrink, H. G. K., and Goudsmit, J.: The Determination of Aneurin (Vitamin B_1) in Urine by the Thiochrome Reaction, Rec. trav. chim. **56:** 803, 1937.

[53] Pyke, M. A.: The Chemical Measurement of Vitamin B_1 in Foodstuffs and Biological Material by Means of the Thiochrome Reaction, Biochem. J. **31:** 1958 (Nov.) 1937.

Karrer and Kubli[54] also give a detailed description of the thiochrome reaction. These investigators feel that the violet fluorescence of thiochrome can be estimated more advantageously by the eye than by a photoelectric fluorimeter. Hennessy and Cerecedo[55] report success in the elimination of nonspecific fluorescence by employing synthetic zeolites for absorbing the vitamin. Recent reports indicate that vitamin B_1-thiamin—and the phosphorylated compound-thiamin pyrophosphoric acid or co-carboxylase—must be determined separately by the thiochrome reaction.[55a]

Several other tests suggested for vitamin B_1 also depend on the formation of colored compounds. Prebluda and McCollum[56] described a purplish red compound formed with vitamin B_1 and *p*-amino acetanilid or methyl-*p*-amino phenyl ketone treated with nitrous acid which was stable and highly insoluble in water. Naimans[57] found that with vitamin B_1 containing substances bismuth potassium iodide gives an orange red precipitate which can be filtered, dried and weighed. This reaction is specific for the thiazole nucleus and the weight of the precipitate is proportional to the quantity of vitamin B_1 present. Raybin reported the formation of an orange solution when vitamin B_1 reacted with 2-6 dibromo-quinone-chloro-imide. The colored compound was extractable by immiscible solvents.[57a]

In 1926 Jansen and Donath claimed a strong Pauly reaction (red color when treated with diazotized sulfanilic acid in the presence of sodium carbonate) for crystalline preparations of vitamin B_1. This reaction was studied by Kinnersley and Peters,[58] who found that it was stabilized by the addition of formaldehyde. These investigators felt, however, that the specificity of the reaction needed further proof.

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[54] Karrer, Walter, and Kubli, Ulrich: Zur Bestimmung von Vitamin B_1 (Aneurin), Helv. chim. acta. **20**: 369, 1937.

[55] Hennessy, D. J., and Cerecedo, L. R.: The Use of Synthetic Zeolites in the Estimation of Vitamin B_1 by the Thiochrome Reaction, read before Division of Biological Chemistry, American Chemical Society, Dallas, Texas, during 1938.

[55a] Westenbrink, H. G. K., and Goudsmit, J.: Quantitative Measurement of Vitamin B_1 by the Thiochrome Reaction, Nature **142**: 150 (July 23) 1938. Ochoa, S., and Peters, R. A.: Quantitative Measurement of Vitamin B_1 and Its Phosphoric Esters and Their Synthesis in Animal Tissues, Nature **142**: 356 (Aug. 20) 1938.

[56] Prebluda, H. J., and McCollum, E. V.: A Chemical Reagent for the Detection and Estimation of Vitamin B, Science **84**: 488, 1936. Melnick, D., and Field, H.: Studies on the Chemical Determination of Vitamin B_1. Proc. of the Amer. Soc. of Bio. Chem., J. Biol. Chem. **123**: lxxxiii (May) 1938. Melnick, D., and Field, H.: Use of Phenol in Application of Prebluda-McCollum Reagent for Determining Vitamin B, Proc. Soc. Exper. Biol. & Med. **38**: 723 (June) 1938.

[57] Naimans, Barnet: A Reagent for Vitamin B_1, Science **85**: 290 (March 19) 1937.

[57a] Raybin, H. W.: A New Color Reaction of Vitamin B_1 (Thiamin), Science **88**: 35 (July 8) 1938.

FOOD SOURCES

Vitamin B_1 has been shown to be present in a wide variety of foods, but there are few foods of plant or of animal origin that may be considered potent sources of this factor. In planning adequate diets, therefore, it is necessary to depend on several food items to furnish the day's supply of vitamin B_1. This is in direct contrast to experience with vitamin A and vitamin C, in which a single food may furnish the major portion of the requirement for the day. The problem of planning for vitamin B_1 is further complicated by the fact that it is desirable to insure an adequate supply in the diet each day, since vitamin B_1 is not stored in the body to any great extent. These considerations become more significant when viewed in the light of the current tendency to give more attention to the subacute and chronic forms of the deficiency diseases.

At the present time dietary calculations for vitamin B_1 are made difficult by the dearth of data on the occurrence of this factor in foods, as well as by the further fact that values expressed in terms of the rat growth unit still comprise the major portion of those available. It is to be hoped that the development of new methods of assay that are more specific and more precise will furnish the incentive for more extensive studies giving values in international units. Tables of numerical values are given in several textbooks, and a summary of data that appeared in the literature up to January 1936 will be found in a publication recently issued by the United States Department of Agriculture.[59] It seems unnecessary, therefore, to consider here more than the general aspects of food sources of vitamin B_1 with particular reference to the precautionary measures essential to insure against unnecessary losses during manipulative procedures, such as cooking or canning.

Vegetables, including potatoes, may be regarded as forming one of the more important groups to be depended on regularly for vitamin B_1. Exclusive of legumes they may be considered for general purposes on a par with one another, since they do not show differences among themselves that make it feasible to group them for relative B_1 content on the basis of any particular physical characteristic such as color, for instance. Although fruits may be rated as only fair sources of vitamin B_1, they are important in that they are generally eaten raw and in relatively large quantities, if used at all.

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[58] Kinnersley, H. W., and Peters, R. A.: The Formaldehyde-Azo Test for Vitamin B_1, Biochem. J. **28**: 667 (No. 2) 1934.

[59] Daniel, E. P., and Munsell, H. E.: Vitamin Content of Foods, Miscellaneous Publication 275, U. S. Department of Agriculture.

Vitamin B_1 tends to be concentrated in the germ portion and outer or bran layer of seeds. Legumes, nuts and whole grains are among the good food sources. Refined cereals and flours, however, contain very little of the vitamin because the germ and the bran layer are largely removed during the refining process.

Eggs, muscle meats and, more especially, the organ meats are rated as good sources. These products are not subject to variation due to changes in the vitamin B_1 content of the diet of the animals from which they are derived, as is the case with vitamin A. Certain muscle meats, specifically chicken and pork, have been described as rather unusual sources when compared with other similar products. Whether this is actually the case or only an accident of assay should perhaps be left for further investigation.

Milk becomes an important source of vitamin B_1 when considered on the basis of the quantity usually consumed and the fact that it may be used without treatment that would tend to lessen its potency.

The foregoing statements should be taken as applying to untreated or fresh forms of the products described. They do not cover foods that have been subjected to any of the various manipulative procedures concerned in preserving the food or preparing it for consumption. Vitamin B_1 is inactivated by heat in the presence of moisture, but in foods at least it is not readily destroyed through oxidation.

The actual loss of vitamin B_1 during the heat treatment of food may or may not be significant, depending on the conditions that prevail. Under most conditions inactivation occurs gradually as the time or temperature, or both, of heating increases. For general purposes this need not be considered significant up to one hour at 100 C. The acidity (or alkalinity) of the medium, however, has a significant influence on the rate of destruction, since inactivation increases with decreasing acidity, especially at temperatures above the boiling point of water. Inactivation of vitamin B_1 in milk powder during dry heating in air at 100 C. for as long as forty-eight hours has been reported as small. Recent experience[60] indicates, however, that losses in foods under conditions comparable to those in baking may be as high as 50 per cent.

Although vitamin B_1 is not readily destroyed at the temperature of boiling water, the greatest loss due to cooking may take place in foods that are boiled. Vitamin B_1 is very soluble in water and it is easily conceivable that a large proportion of the vitamin may go into solution in the cooking water and be lost. Unless this liquid is served in some way to the person eating the food, the food itself should be held a questionable source of vitamin B_1, even though in the untreated form it is rated as a good source. This illustrates a cardinal point that unfortunately is often overlooked, namely that it is not the inclusion of a food item in the diet that is important but its actual nutritive value when consumed.

During canning of foods there is probably little loss of vitamin B_1 due to processing per se. Any loss noted in such products probably took place during the preparation of the food prior to the time it was put in the can. The full vitamin B_1 value of canned products is obtained only if no portion of the contents is discarded.

Too few data are available on the effect of storage on the vitamin B_1 in foods to admit of any definite generalization in this regard. There is little reason for believing, however, that there is any significant loss of vitamin B_1 under storage conditions that preserve the food satisfactorily for consumption. Information on the effect of drying is also scanty. The foods that have been assayed in the dried state were, for the most part, dried to reduce the bulk of the sample so that the experimental animal would consume the portion allotted. The material was dried under conditions assumed to effect no loss of vitamin B_1. This was only an assumption, however, since check tests could not be made on comparable samples of the fresh product. Results of assays carried out in this way have little significance, therefore, in relation to the vitamin B_1 content of the fresh product. Milk may be cited as an exception, since studies designed to determine losses of vitamin B_1 during procedures used to reduce the water content have been made on comparable samples of the fresh and dehydrated or partially dehydrated product. Losses during evaporation and drying were shown to be small.

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[60] Unpublished data, Bureau of Home Economics, U. S. Department of Agriculture.

Freezing seems to be a satisfactory method of preserving the maximum nutritive value of foods and there is reason to believe that vitamin B_1 is preserved in stored frozen foods, as well as are the other vitamins. When frozen foods are cooked, it is essential to remember that the vitamin may be more easily extracted from these than it is from the fresh product, and that care must be taken to serve all expressed juices.

Although it is permissible to claim that the ordinary diet may easily contain adequate supplies of vitamin B_1, it seems advisable to suggest that there is an almost equal opportunity for it to be low in this factor. The tendency to replace whole grain products by refined cereals and flour may entirely eliminate this important source for many individuals. Vegetables are often cooked with little or no regard for loss of vitamin B_1 value or, for that matter, nutrients of any sort. These two indiscretions alone eliminate two of the chief sources of this vitamin. Fruits, meat, milk and eggs, which constitute good sources of vitamin B_1, are among the high cost foods and are the first to be omitted in the low cost diet. With these considerations in mind it would seem to be expedient to give more attention to the vitamin B_1 supply especially in certain types of diets and to the possible ill effects that may result from the long-continued use of a diet low in this nutritionally important factor.

CHAPTER XII

HUMAN REQUIREMENTS FOR VITAMIN B_1

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Other papers in this series discuss the chemistry of vitamin B_1, the pathology of vitamin B_1 deficiency and the physiologic function of this dietary essential. What can be said concerning the amount of this vitamin needed by man?

As beriberi is due to lack of vitamin B_1, it is natural that the earliest estimates of the human requirement of this factor should have been in terms of the therapeutic value of certain foods. For example, Hulshoff-Pol[1] studied the efficacy of katjang idjo, a native bean (Phaseolus radiatus), in the cure of beriberi. The practical result of this type of investigation was to focus attention on the importance of foods containing vitamin B_1 and therefore their inclusion in the dietary. All work along this line prior to about 1911, when Funk[2] introduced the word "vitamine," necessarily suffered the disadvantage that controversy still prevailed respecting the exact cause of beriberi and therefore the validity of any dietary measures in its treatment. In 1907 Braddon[3] published his impressive monograph "The Cause and Prevention of Beriberi," in which he championed the idea that subsistence on a white rice diet is responsible for beriberi, but explained the disease as due to a toxin present in the rice. Although this explanation was later shown to be erroneous, Braddon's book has rendered a great service to students of beriberi because it contains a vast amount of information relating to beriberi epidemics. This material was reexamined in the light of the deficiency disease hypothesis by

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[1] Hulshoff-Pol, D. J.: Katjang Idjo, un nouveau médicament contre le beri-beri, Janus **7**: 524-534, 1902.

[2] Funk, Casimir: The Chemical Nature of the Substance Which Cures Polyneuritis in Birds Induced by a Diet of Polished Rice, J. Physiol. **43**: 395-400, 1911.

[3] Braddon, W. L.: The Cause and Prevention of Beriberi, New York, Rebman Limited, 1907.

Vedder,[4] whose book appeared almost immediately after the word "vitamine" had been suggested by Funk. When it had become clear that beriberi is fundamentally a vitamin deficiency and that it is possible to study the protective factor in experimental animals, a new kind of evidence[5] bearing on the question of the human need for this vitamin appeared in the scientific literature, evidence based on comparisons of the requirements of different species of animals and their suggestions for the human species. For the ideal type of information needed to answer the question of the human requirement for this vitamin it was of course necessary to await isolation of the factor in pure form and tests of it on the human species, something accomplished only recently.[6] In the light of this development of the subject it must be obvious that it has not as yet been possible to make as many tests with the pure vitamin as might be desired, but that these may reasonably be expected in the near future.

From Osborne and Mendel's[7] quantitative experiments in 1922, designed to determine the rat's need for vitamin B, it became clear that the requirement for this factor increases with increase in body weight. In 1923 I was much impressed with the fact that, per unit of body weight, the rat was found to require roughly five times more vitamin B (or vitamin B_1, according to the newer terminology) than the dog. This suggested that man, a still larger species, might be found to require relatively even less than the dog. From what was known at the time regarding the adequacy of human diets and the rat's need for vitamin B_1 it was evident that man could not possibly require relatively as much as the rat. It occurred to me that quantitative experiments on as many species as possible might lead eventually to knowledge applicable to man. Such experiments were therefore made on four species, the mouse, rat, pigeon and dog. All of these tests were made with the same vitamin B_1 concentrate in order to avoid error due to the presence of unknown dietary essentials, the amounts of which might vary in different materials. Preliminary observations of cures of animals suffering from advanced B_1 deficiency gave such variable results that it was decided to make the desired quantitative tests on animals as nearly normal as possible. Therefore the prevention of the characteristic anorexia which appears in response to lack of vitamin B_1 was made the physiologic sign of interest.

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[4] Vedder, E. B.: Beriberi, New York, William Wood & Company, 1913.

[5] Cowgill, G. R.: The Vitamin B Requirement of Man, New Haven, Yale University Press, 1934.

[6] Williams, R. R.: The Chemistry of Thiamin (Vitamin B_1), J. A. M. A. **110**: 727 (March 5) 1938.

[7] Osborne, T. B.; Mendel, L. B., and Cannon, H. C.: Quantitative Aspects of the Role of Vitamin B in Nutrition, J. Biol. Chem. **54**: 739-752 (Dec.) 1922.

When the minimum dosages of vitamin for a given species were plotted on coordinate paper against body weight, a curve resulted. Attempts to secure a straight line finally resulted in the discovery that the dosage of vitamin was in an approximately linear relationship to the body weight raised to the five-thirds power. This first approximation type of relationship was found to hold quite well within the ranges of body weight studied in the four species, a fact which naturally suggested that it would also hold for the human species. It was further observed that the values of the equating constant (K_{vit}), derived from the plots of data for the various species, were in some way related inversely to the "average maximum size" of the species, the mouse requiring the most and the dog the least per unit of body weight, with the rat and the pigeon in appropriate intermediate positions.

Obviously it was necessary to determine in more detail the nature of this inverse relation to body size if the human requirement was to be found. This was finally achieved with accuracy sufficient for the purpose by comparing the various species on the basis of a new variable called the maximum normal size of the species. When this was done it was possible to place all the species on the same straight line with respect to the vitamin B_1 requirement. It was then evident that a value for the human requirement would be suggested by the "maximum normal weight" for man. Data on weight were secured from actuarial tables and a formula then constructed by which to express man's need for vitamin B_1.

The next step in the investigation consisted of actual tests of this expression to determine its validity and limitations, if any. In order to do this it was necessary (1) to estimate as accurately as possible the vitamin B_1 content of various diets known to have been associated with the presence or absence of beriberi and

(2) to apply the new formula to the persons who ate those diets. This is hardly the place to review the results of the study in any detail; the interested reader is referred to the monographic report.[5]

Let it suffice to point out that examination of numerous diets known to have been associated with epidemics of beriberi revealed that their contents of vitamin B_1 were indeed too small to have met the estimated needs of the persons subsisting on them; therefore beriberi should have appeared, as it did. Likewise, numerous diets known to have been associated with absence of beriberi were found to contain amounts of the vitamin above those estimated to have been required by the persons eating them. These calculations naturally required some knowledge of the vitamin B_1 content of numerous foods, and a large body of information was obtained indirectly from consideration of assay data in the literature. It is worthy of note that a similar study of beriberi diets made by Baker and Wright,[8] using for the calculations the results of food assays made by the same method in the same laboratory, has yielded essentially the same results. Another confirmation has come out of the work of Van Veen[9] carried out in Java.

In this connection it is pertinent to point out that I have always considered the formula derived from this research as constituting merely a first approximation. In the final chapter of my monograph I particularly invited the cooperation of workers everywhere in the confirmation or determination of limitations of these results and the deductions drawn from them. Considering the indirect nature of the approach to the problem, the necessity for making numerous assumptions, and the possibilities resident in further researches in this field, it would be most surprising indeed if the results of this first approximation study should not require modification as a result of later discoveries. particularly those made possible by the isolation and ready availability of the pure vitamin.

Certain difficulties in my work, to which a critic might call attention, have always been appreciated. For

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8. Baker, Audrey Z., and Wright, Margaret D.: Vitamin B_1 in Human Diets, Proc. Roy. Soc. Med. **29**: 1145-1154 (July) 1936.

9. Van Veen, A. G.: Het B_1-type gehalte van veodingsmiddelen (summary and discussion given in English on last two pages), Geneesk. tijdschr v. Nederl.-Indië **75**: 2050-2064 (Nov. 26) 1935.

example, in the rat the five-thirds power relationship, strictly speaking, applies only to animals ranging from about 70 to 150 Gm. in body weight, for the data obtained and discussed were yielded by animals of this size. It may be a quite unjustified extrapolation to apply the formula to larger animals. However, it is certainly significant that when Drs. W. H. Eddy and M. Kellogg repeated the study on rats they found precisely the same relationship to hold with animals up to about 150 Gm. weight; with larger animals, however, the formula did not hold. It may be that the formula stands in some relation to growth, or more particularly the changing caloric exchange characteristic of growth, and this idea receives support from the work of Hendricks.[9a] On examination of the caloric intakes of rats of different weights, taking for example the food intake data of Osborne and Mendel cited in my monograph (on page 82), one obtains over the range of weights covered by these vitamin B experiments a curve similar to the ordinary growth curve, but after about 150 Gm. of body weight the plot of caloric intake against weight proves to be practically a straight line with caloric intake increasing slowly with increasing body weight. In view of these considerations it is quite possible that the five-thirds power relationship set forth in the vitamin formula does not apply to these large rats but that some other relationship holds. The same point of view applied to the dog may help to explain why the five-thirds power formula gives as the quantity of vitamin B required by a large Newfoundland dog, for example, an apparently altogether too large amount.

I am also aware that my plot of species vitamin constant against maximum normal weight of the species involves a plot of logarithms against logarithms, and therefore the points actually plotted may represent appreciably variable instead of quite fixed values. Even in the face of this fact it appears obvious to me that one still obtains as the outstanding feature of the resulting plot a definitely inverse relationship of species vitamin B_1 requirement to maximum normal size of the species. Furthermore it seems especially significant that it was from this particular plot that a suggestion was obtained of the appropriate constant to use in the formula applicable to man; and tests of the formula thus derived on various human diets have given essentially the same results as those yielded by other workers who have used more recent and exact data on vitamin contents of foods, as well as experimented with the pure vitamin. It is difficult to believe that such an outcome and confirmation of the formula can be merely fortuitous.

[9a] Hendricks, W. A.: The Relation of Vitamin B Requirement to Metabolism, Am. J. Physiol. **105:** 678-683 (Sept.) 1933.

The formula (see table) indicates that the value of the vitamin B_1 : calory ratio increases with increase in body weight. In a personal communication Dr. R. R. Williams has expressed his disagreement with this point. He feels that the fundamental relationship is merely between vitamin B and carbohydrate or non-fat calories. In the light of the newer researches indicating a probable role of this vitamin in the metabolism of carbohydrate,[10] such a view is well supported. However, the question as to whether the critical value of the vitamin : calory ratio increases with increase in body size can hardly be disposed of in such simple fashion in my opinion. With the perfection of chemical methods for analysis of blood and urine for vitamin B_1 and their application in studies of vitamin B_1 physiology, it should be quite possible sooner or later to obtain data bearing definitely on this question. From certain preliminary observations which Jolliffe and collaborators have shown me it is reasonable to believe that the value of this ratio may indeed prove to vary with body weight as I have already suggested. For all practical purposes and the evaluation of human dietaries the settlement of this question may be quite academic. The final answer will probably be obtained only when experiments specially designed for the purpose have been performed.

The formula by which to estimate a given person's requirement for vitamin, as stated originally, expressed the vitamin variable in terms of the milligram-equivalent, a unit based on the particular yeast concentrate used in the studies from which the formula was derived. It is of course more desirable that the international unit be used, and a table giving factors for converting the milligram-equivalent to the international, Sherman and other units was included in the monograph describing the formula. From a theoretical point of view the ideal unit is a given amount of the pure crystalline vitamin, i. e., thiamin chloride, and it is interesting, therefore, to learn that the international committee has finally adopted such a standard [10a] and has redefined the international unit as the potency of 3 micrograms of the pure substance. In view of this it becomes possible to state the value to be given K_{vit} in my formula in order to obtain the requirement in terms of the new international unit (see table). Because other units will doubtless continue to be used during a transition period of adjustment to the new one, values to be used when these other units are involved are also given.

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[10] Cowgill, George R.: The Physiology of Vitamin B_1, J. A. M. A. **110**: 805 (March 12) 1938.

It should be emphasized that estimates of the human requirement for vitamin B derived from my formula pertain to the minimum or beriberi-preventing level; the optimal intake is undoubtedly much greater. What should be taken as the optimal requirement will be discussed later.

Table for Obtaining Vitamin Values

When vitamin is expressed in terms of the units indicated below, the value of K_{vit} to use in the formula $\frac{\text{vitamin}}{\text{calories}} = K_{vit} \cdot \text{weight}_{\text{kilogram}}$ is that shown in the table.

Unit of Vitamin B	K_{vit}
Milligram-equivalent (Cowgill[5])	0.0284
International unit (1934)	0.00142
International unit (1938): 1 unit equal to 3 micrograms of thiamin chloride	0.00426

THE NORMAL ADULT

What does the formula suggest as the minimum vitamin B_1 intake necessary for the adult human being? Obviously the weight is significant. Also, as the formula indicates, the vitamin requirement is related to the caloric intake, and, in view of what is known about the probable function of the vitamin in the body,[10] a still closer approximation would be with respect to the calories from carbohydrate metabolism. Assuming an average degree of activity and therefore level of daily energy intake, it appears that a man weighing 45 Kg. (99 pounds) requires as a minimum about 135 interna-

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10a. Cablegram from Dr. Harriet Chick of London, Secretary of the International Vitamin Conference, to the Vitamin Advisory Committee of the U. S. Pharmacopoeia in Session in New York City, July 22, 1938; Memorandum on the Second International Standard Vitamin B_1, League of Nations Bull. of the Health Org. **7:** 882-886 (Oct.) 1938.

tional units of the vitamin; a person weighing 70 Kg. (154 pounds) needs approximately 280 international units, and a still heavier person weighing 90 Kg. (198 pounds) requires about 550 international units. Obviously the exact figure depends very much on the number of calories involved or taken in the calculation.

These values agree quite well with those suggested by other students of the problem. Thus Jansen, working with the small stature races of the Far East, estimated that from 1 to 2 Gm. of the international standard adsorbate was required daily as a minimum, with about 4 Gm. constituting a curative dose for patients with beriberi. Since 1 international unit is defined as the activity of 10 mg. of the standard adsorbate, his dosages can be translated to from 100 to 200 international units as the daily minimum and 400 international units for the cure of beriberi. These minimums, pertaining to people with body weights ranging from about 45 to 60 Kg., obviously agree very well with those suggested by the formula.

Reference has already been made to the relationship of the vitamin requirement to the caloric intake. As it is easy to estimate the number of calories yielded by a given diet, a simple form of standard to use in expressing the vitamin need is the number of units of vitamin per hundred calories. Stated in this way the minimums cited mean approximately 10 international units per hundred calories. Rose [11] has examined the reports of numerous investigators and recommended a daily intake of 30 Sherman-Chase units per hundred calories as a liberal allowance, an intake which would be equal to 15 international units per hundred calories. As pointed out by Daniel and Munsell,[12] "for the average adult this would mean between 750 and 900 Sherman-Chase units, or roughly 250 to 400 international units per day, quantities admittedly well above the minimum requirement."

Vorhaus, Williams and Waterman [13] estimated that the normal adult takes in daily about 1 mg. of pure vitamin B_1; they did not mean that this necessarily represents a definite requirement, however. If one assumes that from 3 to 5 micrograms of the pure substance equals 1 international unit, this estimate means a daily intake of from 333 to 200 international units, which is in the range of minimums previously suggested.

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[11] Rose, Mary S.: The Foundations of Nutrition, New York, Macmillan Company, 1933.

[12] Daniel, Esther, and Munsell, Hazel E.: Vitamin Content of Foods: A Summary of the Chemistry of Vitamins, Units of Measurement, Quantitative Aspects in Human Nutrition and Occurrence in Foods, Miscellaneous Publication 275, U. S. Department of Agriculture, June, 1937.

[13] Vorhaus, M. G.; Williams, R. R., and Waterman, R. E.: Studies of Crystalline Vitamin B: Experimental and Clinical Observations, J. A. M. A. **105**: 1580-1584 (Nov. 16) 1935.

Evidence of another type bearing on this question may be cited in the work of Harris and Leong.[14] These investigators studied the excretion of vitamin B_1 through the kidney. The healthy adults studied by them, from 13 to 37 years of age and subsisting on normal diets, were found to excrete daily from 12 to 35 international units, or from 30 to 90 micrograms of the pure vitamin hydrochloride; this excretion was estimated to be approximately from 5 to 8 per cent of the total daily intake of the vitamin. These authors concluded that a urinary output of vitamin B_1 below 12 international units daily, or approximately 1 unit per hundred cubic centimeters, should be regarded as evidence for strongly questioning the adequacy of vitamin B_1 in the diet. On the basis of these observations the minimum daily standard for a man weighing 140 pounds (63.5 Kg.) was set at 200 international units. It is evident that this estimate of the minimum agrees well with the others which have been cited.

Further data bearing on this question may be cited. Van Veen[9] has suggested 150 international units per day as the beriberi-preventing minimum suited for the inhabitants of the Dutch East Indies. From their studies of numerous dietaries throughout the world Baker and Wright[8] of London arrived at from 200 to 500 international units per day as the minimum for man. It seems pertinent to point out that the oriental races represented by the Van Veen report are normally of smaller stature than Europeans and Americans. The lower suggested minimum of Van Veen might be interpreted in the light of my formula as due to the vitamin: calory ratio having a lower value for these people of smaller body weight. Against this view, of course, it can be argued that these people eat less food and the fundamental relationship is really between the vitamin and the amount of food or number of calories ingested.

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[14] Harris, L. J., and Leong, P. C.: Vitamins in Human Nutrition: The Excretion of Vitamin B_1 in Human Urine and Its Dependence on the Dietary Intake, Lancet **1**: 886-894 (April 18) 1936.

A still more recent contribution is seen in the work of a German group[14a] of investigators, who have suggested that the minimum for man is from 250 to 750 micrograms of thiamin per day. Since there is no unanimity of opinion regarding the number of micrograms of thiamin to be taken as equivalent to 1 international unit, it is difficult to say exactly how many international units these suggestions represent. Taking 1 international unit as equivalent to from 2 to 5 micrograms of thiamin, the range covered in the table, the lower figure (250 micrograms) might be from 50 to 125 international units; similarly the larger figure (750 micrograms) could be anywhere from 150 to 375 international units. What is probably more significant is the suggestion of these German authorities that from 1 to 2 milligrams of pure thiamin daily is needed for health, which agrees with the opinion of Williams and associates.

The perfection of chemical methods for analyzing the urine and other biologic fluids for vitamin B_1 should lead to further extensive studies to determine the extent to which an injected standard dose of the pure vitamin is excreted through the kidney and the relation of the amount excreted to the store in the tissues. If the tissue reservoir is not saturated, it is reasonable to suppose that a smaller fraction of the injected vitamin will be eliminated in a standard interval of time; with the tissues already storing the maximum amounts, greater portions of the injected test dose should be eliminated. Perfection of the technic for such a test would mean that a valuable clinical function test for vitamin B_1 deficiency had been obtained.

Such an "injection-saturation-excretion technic" has already yielded valuable results in studies of the body's requirement for vitamin C. It is of course possible that the same results would not be obtained with thiamin because of differences in function in the body, etc. Some preliminary observations, which Dr. N. Jolliffe and associates have shown me, suggest that this may after all prove to be the case, that vitamin B_1 differs from vitamin C in respect to tissue saturation and elimination. The entire subject merits extended study which should lead to results useful both to public health and hygiene and to clinical medicine.

[14a] Stepp, W.; Kuhnau, J., and Schroeder, H.: Die Vitamine, Stuttgart, F. Enke, 1937, p. 42.

MOTHER AND INFANT

What is known about the vitamin B_1 requirement of the human mother and her infant? The high incidence of infantile berberi in certain regions is evidence of the importance of this question. In this connection it is important to bear in mind two facts: 1. During the first six months of its life the human infant subsists almost entirely on milk and is therefore in the category of persons who live on a restricted dietary. 2. The amount of vitamin B_1 present in the milk has been shown to be dependent primarily on the quantity ingested by the mother. Studies on rats[15] have shown that for a lactating rat to nurse her litter successfully the vitamin B_1 intake must be about five times the amount needed for mere maintenance of the maternal organism. Studies of numerous dietaries[16] used in regions where beriberi is endemic have shown that they are low in vitamin B_1 content. For example, Baker and Wright[16] found the B_1 content of a group of such dietaries to range from 71 to 180 international units daily. It is not surprising, therefore, that the lactating women of many sections of the Far East who subsist on these faulty diets should secrete milk containing too small an amount of the vitamin for the infant and that infantile beriberi should be of common occurrence in these regions.

In 1935 the Technical Commission of the League of Nations Health Committee published a report entitled the "Physiological Bases of Nutrition,"[17] dealing particularly with the vitamin requirements of pregnancy and lactation and recommending during these two physiologic states a daily vitamin B_1 intake of from 150 to 250 international units. This suggestion is based on daily intakes of from 3,000 to 3,500 calories during lactation. In the light of the preceding discussion such a recommendation seems altogether too low. It is of course difficult to make any all-inclusive single statement as to what the human mother should consume daily (this is what the commission was attempting to do) because the factors which must be considered do not always operate to the same degree in every person. In lactation the body weight, the total caloric exchange and the volume of milk secreted all have a part as well as the intake of vitamin. It is possible that the recommendation of the commission may be regarded as reasonably satisfactory for the average woman of certain races normally smaller in stature than our own. For the American mother, however, I believe the daily vitamin B_1 intake might well be not less than 15 and preferably as high as 20 international units per hundred calories. If for any reason it is difficult to secure these higher levels of intake, obviously the vitamin may be given directly to the infant.

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[15] Evans, H. M., and Burr, G. R.: On the Amount of Vitamin B Required During Lactation, J. Biol. Chem. **76:** 263-272 (Jan.) 1928. Sure, Barnett: Dietary Requirements for Fertility and Lactation: XV. The Inefficiency of the Lactating Mother (Mus Norvegicus Albinus) in Secreting Vitamin B in the Milk, and the Relation of This Phenomenon to Infant Mortality (detailed report), ibid. **76:** 685-700 (March) 1928.

[16] Baker, Audrey Z., and Wright, Margaret D.: Vitamin B_1 Content of Human Diets, Brit. M. J. **1:** 599-600 (March 21) 1936. Cowgill.[5]

[17] League of Nations Health Committee, Technical Commission: Report on Physiological Bases of Nutrition, London, 1935.

More exact data bearing on this question might be secured from a study of the effects in lactating women of various levels of vitamin B_1 intake on the concentration of the vitamin in the milk secreted; the intake giving the highest concentration of vitamin in the milk and above which no further increase is produced might be taken as the standard of vitamin B_1 intake during lactation in the human species. Now that the pure vitamin is available, it should be possible to perform experiments with the desired limitation of variables not possible hitherto. Studies of the maximum retention of doses of pure vitamin administered to lactating women differing markedly in size and caloric intake should yield data useful in relation to this question.

This problem may also be considered from the standpoint of the vitamin intake of the nursing infant. The "American Public Health Year Book" for 1934-1935 [18] states that the daily requirement for vitamin B_1 increases from 50 to 200 international units between infancy and adolescence. Should this lower figure, 50 international units, be regarded as a suitable optimum for the month old infant? If one considers the baby's daily caloric intake of human milk having the maximum amount of vitamin B_1 ever found, how much vitamin B_1 would the infant receive from such milk? My calculations give a figure approximately 80 international units instead of the 50 stated in the Year Book's recommendation. Until extensive quantitative studies have been made and this result shown to be erroneous, I suggest that 80 international units daily be taken as the optimum for the very young baby. One might perhaps call this a minimum, by which one would mean that no baby should receive less than this amount daily.

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[18] American Public Health Year Book 1934-1935: Human Requirements for Vitamins, pp. 69-72.

From theoretical considerations one should expect the vitamin B_1 requirement to be greater for growth than for maintenance. In addition to the vitamin consumed during the metabolism associated with mere maintenance of the organism, the growing person needs the vitamin which is to become a normal part of the new cells produced by growth. Furthermore, since the caloric exchange and the general level of energy metabolism are relatively higher during growth, one may also expect to find a higher vitamin B requirement for growth because of this increase in the variable in the formula representing metabolism. Dann and I [19] studied this question in the rat by comparing the amount of vitamin B_1 needed to secure the good growth characteristic of our colony with the amount required for maintenance at a given body weight after the rats had made good growth to that weight. The data indicated that the B_1 requirement for good growth in the female rat is from three to five times the need for mere maintenance.

THE CHILD

What is known about the child's need for vitamin B_1? The formula discussed in the first part of this paper applies more strictly to the adult organism and gives only the beriberi-preventing minimum. Theoretically, children subsisting on a diet just sufficient for their parents should not acquire beriberi until by growth they have reached the approximate size of the parents. The primary effect of such a diet on the children would probably be some limitation of growth. This would explain why beriberi, although common among breast-fed infants of the Far East, is comparatively rare in children between the weaning age and early adult life. It becomes appropriate, therefore, to examine the growth of children in relation to the supply of vitamin B_1.

It appears evident from the reports of several investigators that shortage of this vitamin has operated in part to limit the growth of children in North America. For example, Morgan and Barry,[20] in their study of underweight children, obtained weight gains from 150 to 170 per cent above the expected gain when they fed two wheat germ rolls at the noon meal; these gains may be contrasted with the gains of the control group, which received white flour rolls and made only from 50 to 71 per cent of the expected gain during the same period. Summerfeldt[21] observed gains of 4.54 and 5.27 pounds (2 and 2.4 Kg.) in ten weeks associated with the administration each week of 4.2 ounces (113 Gm.) of wheat germ and 0.28 ounce (8 Gm.) of yeast, these supplements being incorporated in a special breakfast cereal; the control groups, receiving ordinary cereals, gained only 1.25 and 1.27 pounds (567 and 576 Gm.), respectively. An extension of this study was made by Ross and Summerfeldt,[22] who also used a vitamin B_1 concentrate in addition to the ordinary cereal; their results indicated that the gain with the special cereal was in large measure due to its vitamin B_1 content. The observations of Bartlett[23] and Schlutz[24] of anorexia in children brought to an outpatient clinic suggest that lack of vitamin B_1 is more common than is generally appreciated.

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[19] Dann, Margaret, and Cowgill, G. R.: The Vitamin B Requirement of Female Albino Rats for Maintenance and Growth, Am. J. Physiol. **109**: 27-28 (July) 1934.

Knott[25] made vitamin B_1 balance studies with eight children from 4 to 7 years of age. "In the . . . study, the intakes which resulted in the highest retentions (regardless of whether retentions were calculated on the basis of food minus urine or food minus total excretion) were found to be six to seven times greater than the minimum requirement for preventing beriberi as determined by means of the formula suggested by Cowgill." Knott also pertinently remarked: "If the intakes resulting in highest retentions may be considered optimum, this wide range between minimum and optimum requirement would seem to explain both the existence of vitamin B deficiency among children and the beneficial results obtained by additions of vitamin B to the diet." This suggested optimum for young children was expressed by Knott as "about 40 Chase-Sherman units per kilogram per day." This would be equal to about 20 international units per kilogram daily. On the basis of the data on body weight and caloric intake recorded by Knott, this figure may be translated to mean from about 20 to 25 international units per hundred calories.

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[20] Morgan, Agnes F., and Barry, Margaret M.: Underweight Children: Increased Growth Secured Through the Use of Wheat Germ, Am. J. Dis. Child. **39**: 935-947 (May) 1930.

[21] Summerfeldt, Pearl: The Value of an Increased Supply of Vitamin B_1 and Iron in the Diet of Children, Am. J. Dis. Child. **43**: 284-290 (Feb.) 1932.

[22] Ross, J. R., and Summerfeldt, Pearl: Value of Increased Supply of Vitamin B_1 and Iron in the Diet of Children, II, Am. J. Dis. Child. **49**: 1185-1188 (May) 1935.

[23] Bartlett, W. M.: An Analysis of Anorexia, Am. J. Dis. Child. **35**: 26-35 (Jan.) 1928.

[24] Schlutz, F. W.: The Role of the Diet in the Treatment of Disorders of Older Infants and Children, Arch. Pediat. **42**: 347-362 (June) 1925.

[25] Knott, Elizabeth M.: A Quantitative Study of the Utilization and Retention of Vitamin B by Young Children, J. Nutrition **12**: 597-611 (Dec.) 1936.

CLINICAL FACTORS INFLUENCING THE VITAMIN B_1 REQUIREMENT

Heightened Metabolism.—Examination of the formula given previously reveals that the vitamin B_1 requirement is related to the caloric exchange. If the caloric factor is increased, the body weight remaining unchanged, obviously the vitamin intake must be increased. This rule has been tested experimentally both in dogs[26] and in pigeons[27] and found to be correct. The metabolism factor was increased by administration of thyroid tissue, with consequent production of experimental hyperthyroidism, and in every instance, after the appearance of anorexia, with consequent loss in body weight, the administration of sufficient vitamin B_1 restored the urge to eat and the animals voluntarily ate enough additional food to permit return to the original weight and to secure maintenance at that weight. It may clarify the significance of these results if I point out that the voluntary intake of calories during the experimental hyperthyroidism following administration of vitamin B_1 was practically twice that characteristic of the animals before thyroid was given. In other words, the level of energy exchange induced by the administration of thyroid was approximately double the normal level. In man such a difference would correspond to that seen in a case of extremely overactive thyroid.[28] Although it must be recognized that exophthalmic goiter may not be due simply to an overactive thyroid,[29] it does seem clear that patients suffering from this disease should receive considerably more vitamin B_1 than normal persons. A good practical rule to follow in such cases would seem to be to administer not less than 20 international units of vitamin B_1 per hundred calories of the estimated total daily metabolism. Even this intake would mean using a factor of safety of only 2. If one were to take the measurements of the requirements of pigeons in experimental hyperthyroidism as a guide,[27] a much greater factor of safety would be justified in certain cases. Some of the birds required from two to five times the normal amount of vitamin when given the thyroid. The differences may be related to variation in absorption of material from the alimentary tract and other functions. With the pure vitamin now available, extensive tests of its efficacy in the treatment of exophthalmic goiter should be made; the results of such clinical trials would provide the best basis for the formulation of rules regarding the appropriate dosage of the vitamin.

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[26] Himwich, H. E.; Goldfarb, Walter, and Cowgill, G. R.: Studies in the Physiology of Vitamins: XVII. The Effect of Thyroid Administration upon the Anorexia Characteristic of Lack of Undifferentiated Vitamin B, Am. J. Physiol. **99:** 689-695 (Feb.) 1932.

[27] Cowgill, G. R., and Palmieri, M. L.: Studies in the Physiology of Vitamins: XXII. The Effect of Experimentally Induced Hyperthyroidism on the Vitamin B Requirement of Pigeons, Am. J. Physiol. **105:** 146-150 (July) 1933.

The effect of an increase in the caloric factor on the vitamin B_1 requirement has also been tested in dogs given forced exercise.[30] In every case, increase in the number of calories representing total metabolism was associated with an increase in the vitamin need.

These confirmations of the relationship between calories and the vitamin B_1 requirement suggest to the clinician the wisdom of increasing sufficiently the vitamin B_1 supply in all cases in which there is a significant increase in the energy metabolism. For example, in the treatment of prolonged fevers by the feeding of so-called high calory diets, ample supplies of vitamin B_1 should be given along with the "extra" calories. How much vitamin should be given? It is difficult to make any dogmatic general statements in answer to this question. It can be suggested, however, that some estimate be made of the total daily caloric exchange and not less

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28. Du Bois, E. F.: Basal Metabolism in Health and Disease, Philadelphia, Lea & Febiger, 1936.

29. For a good discussion of the question of the etiology of exophthalmic goiter see Du Bois,[28] p. 348 et seq.

30. Cowgill, G. R.; Rosenberg, H. A., and Rogoff, J.: Studies in the Physiology of Vitamins: XVI. The Effect of Exercise on the Time Required for the Development of the Anorexia Characteristic of Lack of Undifferentiated Vitamin B, Am. J. Physiol. **98**: 589-594 (Nov.) 1931.

than from 15 to 20 international units of the vitamin per hundred calories be provided. If there is a reason to believe that the patient has been for some time on a low vitamin B_1 regimen, the daily administration of from 5 to 10 mg.[31] of the pure vitamin for about a week to build up the tissue reservoir would be wise; afterward the daily intake might well be not less than 15 international units per hundred calories.

Loss Through Excretory Channels.—Vitamin B_1 is a water-soluble substance and presumably, therefore, might be eliminated through the kidneys. Many workers have examined the urine for vitamin B_1 content with positive results. There appears to be little question that the amount of vitamin B_1 present in the urine is affected by the intake and the degree of "saturation" of the tissues.[32] It becomes of interest clinically to know whether vigorous diuresis can operate to affect the organism's need for vitamin B_1 by promoting loss of this dietary factor through the kidneys. That this is indeed possible was shown by the experiment which Rosenberg, Rogoff and I [33] performed on dogs. We observed that mere administration daily of large volumes of water sufficient to produce vigorous diuresis resulted in the appearance of anorexia due to vitamin B_1 deficiency in about one-half the time normally required; control experiments showed that such administration of water together with ample vitamin B_1 did not produce the anorexia.

Many clinical conditions are characterized by pronounced diuresis. It seems clear from these experiments on animals that whenever the clinician encounters pronounced diuresis he should take care to see that the patient receives a sufficiently large extra amount of vitamin B_1 to replace that which may be lost through the kidneys. A factor of safety of at least 2 seems to be warranted. This would mean a daily intake of not less than 20 international units per hundred calories for the average patient; still greater amounts would of course provide a greater insurance against shortage of the vitamin due simply to loss through the kidneys.

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[31] This suggestion is based on the report of clinical trials of the pure vitamin made by Goodhart and Jolliffe.[36]

[32] Papers dealing with this question are cited by Harris and Leong.[14]

[33] Cowgill, G. R.; Rosenberg, H. A., and Rogoff, J.: Studies in the Physiology of Vitamins: XIV. The Effect of Administration of Large Amounts of Water on the Time Required for Development of the Anorexia Characteristic of a Deficiency of the Vitamin B Complex, Am. J. Physiol. **95:** 537-541 (Dec.) 1930.

In the treatment of many clinical conditions it has become standard practice to administer large volumes of fluid. The data at hand from animal experiments suggest the wisdom of administering liberal amounts of vitamin B_1 along with the fluid. The minimum previously suggested based on a factor of safety of 2 might well apply *if the patient is an adult; if he is a child* the results of Knott[25] should be considered and much larger doses given.

Diarrhea is a common feature of many clinical conditions. It has long been known that vitamin B_1 is present in the stools of animals and that the amount present may vary with the composition of the diet.[34] It is theoretically possible that some of the vitamin present in the intestinal excreta came originally from within the organism, having been excreted through the intestinal wall. Two other possibilities are (1) that this vitamin represents the amount in the diet which failed to be absorbed and (2) that it has been produced by micro-organisms, having been either secreted by them into the surrounding medium or present in the stool as part of their bodies. Study of this problem is obviously of some clinical importance. From the experiments which Dann and I[35] performed on dogs it is clear that the effect of diarrhea is to decrease the absorption of an appreciable fraction of the ingested vitamin. In some instances the daily intake by mouth had to be increased as much as 70 per cent above the previous "normal" dose. No evidence was obtained suggesting the loss of previously absorbed vitamin by excretion through the intestinal wall. The diarrhea produced experimentally in our dogs would not be classified as severe; it certainly was not comparable to the pronounced diarrhea characteristic of numerous clinical conditions. If the condition we studied could produce a 50 to 70 per cent loss of ingested vitamin, it is clear that the more severe diarrheas must mean even greater losses. Two methods of treatment with vitamin B_1 suggest themselves; namely, administration by mouth of doses of the order of 20 international units per hundred calories or more, with the expectation of considerable loss due to failure of absorption, and parenteral administration of the pure vitamin in doses not less than about 15 units per hundred calories.

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[34] For a detailed discussion of this point, with citations of literature, see "The Physiology of Vitamin B," this series.

[35] Dann, Margaret, and Cowgill, G. R.: The Influence of Diarrhea on the Vitamin B Requirement, Arch. Int. Med. **62:** 137-150 (July) 1938.

Clinical Trials with Thiamin Chloride.—Some tests with pure vitamin B_1 have already been reported. It is pertinent to my present theme to discuss these briefly.

Reference has already been made to the paper by Vorhaus, Williams and Waterman.[13] These investigators suggested that adult human beings should receive daily about 1 mg. of the pure vitamin. Goodhart and Jolliffe [36] made a study of the efficacy of various doses of thiamin chloride in the treatment of neuritis in alcohol addicts. They observed that the minimum effective dose was of the order of 4 mg. daily; from 10 to 20 mg. gave much more valuable results, and in some cases as much as 50 mg. had to be given in order to secure improvement. Weiss [37] reported tests indicating the value of the 20 mg. dose and the similar interesting observation that some patients, for unknown reasons, require doses as large as 50 mg. in order to show definite clinical improvement. To many clinicians these may seem to be relatively enormous amounts of the pure vitamin to administer. It is pertinent to point out that the results are similar to those obtained with experimental animals. For example, the minimum amount of vitamin B_1 required to cure a pigeon and keep it free from the critical symptoms of advanced B_1 deficiency for at least three days is from three to five times the daily minimum for maintenance of weight. In order to permit rapid restoration to normal body weight and well being, the bird must receive from ten to twenty or more times the daily minimum for maintenance of weight. Assuming that such a relationship holds for man and taking for calculation the daily requirement of the average man as suggested by Vorhaus and his associates,[13] namely, 1 mg. of thiamin chloride, one should expect precisely the results reported by Goodhart and Jolliffe [36] and by Weiss.[37]

In view of what is known about the ready excretion of vitamin B_1 through the kidneys, it seems to me that the clinician must for the present necessarily steer between the one extreme of giving such small daily doses that the patient can improve only very slowly and the other extreme of giving so much (particularly by the parenteral route) that a large part is lost through the kidneys before the tissues can properly absorb and store it. Until studies have been made of the capacity of both the deficient and the normal organism to store vitamin B_1 administered in various ways under properly controlled experimental conditions, one cannot say what are the largest effective yet economical doses of the pure vitamin to use in clinical work. As soon as chemical methods have been perfected, permitting prompt analysis of the urine and the blood for their vitamin B_1 content, these important studies can and should be performed. In view of the activity on this problem in many laboratories, no doubt one may confidently expect soon to have the desired chemical tools for this work. In addition to yielding the information desired with respect to the best doses to use clinically, such studies should also lead to a clinical function test by which the clinician may not only confirm his impression that the patient is suffering from serious vitamin B_1 deficiency but may even detect moderate deficiency, which might be called "vitamin B_1 subnutrition." It is my belief that such researches will reveal vitamin B_1 subnutrition to be sufficiently common to warrant serious attention on the part not only of the practicing clinician but of all students of preventive medicine, public health and nutrition. The condition, if found, could be corrected readily by improvement of the diet.

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[36] Goodhart, R. S., and Jolliffe, Norman: Observations on the Effects of Vitamin B (B_1) Therapy on the Polyneuritis of Alcohol Addicts, J. A. M. A. **110**: 414 (Feb. 5) 1938.

[37] Weiss, Soma: Personal communication to the author.

CHAPTER XIII

CHEMICAL ASPECTS OF RIBOFLAVIN

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WASHINGTON, D. C.

HISTORICAL SKETCH

The chemical nature of the water-soluble yellow green-fluorescent pigment of whey now referred to as riboflavin (synonymous with lactoflavin, vitamin G and vitamin B_2, as used by many English and German investigators) commanded the attention of chemists[1] as early as 1879. A considerable concentration of this pigment was effected and certain of its more obvious chemical properties were clearly set forth by Bleyer and Kallmann[2] in 1925. No unusual significance was associated with this pigment by these early workers, who apparently regarded it only as one of the minor constituents of milk, the chemical nature of which was quite obscure.

In 1932 Warburg and Christian[3] described a new oxidation enzyme obtained from aqueous extracts of yeast. The enzyme in water solutions was yellow and exhibited a green fluorescence. Together with a second enzyme obtained from yeast and a co-enzyme obtained from the red blood cells of horse blood this "yellow enzyme" constituted an enzyme system capable of oxidizing Robison's hexose-monophosphoric acid ester. The "yellow enzyme" was reversibly reduced to its leukoform during the process. To regenerate the pigmented enzyme it was necessary only to shake the solution carrying it in leukoform with molecular oxygen. In this system the "yellow enzyme," by virtue of its easily reversible oxidation-reduction reactions, acts as an oxygen transport between molecular

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From the Bureau of Home Economics, U. S. Department of Agriculture.

[1] Blyth, A. Winter: The Composition of Cow's Milk in Health and Disease, J. Chem. Soc. **35**: 530-539, 1879.

[2] Bleyer, B., and Kallmann, O.: Beiträge zur Kenntnis einiger bisher wenig studierter Inhaltsstoffe der Milch (Kuhmilch) II, Biochem. Ztschr. **155**: 54-79 (Jan.) 1925.

[3] Warburg, Otto, and Christian, Walter: Ueber ein neues Oxydationsferment und sein Absorptionsspektrum, Biochem. Ztschr. **254**: 438-458 (Oct.) 1932.

oxygen and the substrate. It has now been practically established that this "yellow enzyme" is present in every living cell or at least in the cells of all the higher forms of life.[4]

Warburg and Christian [5] were able to separate the "yellow enzyme" from yeast and to split it into a protein component and a pigment component. Neither component alone was catalytically active. The separation of these components was effected by addition of three volumes of methyl alcohol to a water solution of the enzyme at a temperature of 38 C., whereupon the protein was denatured, leaving the pigment component in solution. These brilliant researches constitute the first separation and identification of the prosthetic or chemically active grouping of an enzyme. Irradiation of alkaline solutions of the pigment component followed by acidification of the irradiated solution yielded a yellow chloroform soluble photoderivative (later designated lumiflavin). Studies of the chemical nature of this photoderivative, which can also be obtained readily from the relatively heat-stable vitamin riboflavin, constituted the crux in the elucidation of the chemical structure of the vitamin and of the prosthetic group of the enzyme.

In 1933 there appeared almost simultaneously from three different laboratories reports supporting a relationship and probable identity of the water-soluble yellow green-fluorescent pigments widely distributed in animal and plant products with vitamin G or vitamin B_2, the accepted chemical name for which is now riboflavin. Ellinger and Koschara [6] called attention to the presence of a group of water-soluble yellow green-fluorescent pigments present in milk, liver, kidneys, urine, muscle, yeast and plant materials for which they suggested the name lyochromes, in contradistinction to the group of naturally occurring fat-soluble pigments, lypochromes. These authors suggested also the probable relationship of these pigments to the yellow enzyme discovered by Warburg and Christian. Any one thoroughly familiar with the properties and distribution of the vitamin riboflavin could readily discern from their report the similarity of the qualities of the vitamin with the attributes of the pigments designated as lyochromes.

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[4] Warburg, Otto, and Christian, Walter: Ueber das Gelbe Ferment und seine Wirkungen, Biochem. Ztschr. **266**: 377-411 (Nov.) 1933.

[5] Warburg, Otto, and Christian, Walter: Ueber das neue Oxydationsferment, Naturwissensch. **20**: 980-981 (Dec. 16/23) 1932.

[6] Ellinger, Philipp, and Koschara, Walter: Ueber eine neue Gruppe tierischer Farbstoffe (Lyochrome), Ber. d. Deutschen chem. Gesellsch. **66**: 315-317 (Feb.) 1933; Ueber eine neue Gruppe tierischer Farbstoffe (Lyochrome), ibid. **66**: 808-813 (June) 1933

About the same time I[7] had succeeded in obtaining from whey powder several concentrated growth-promoting supplements for the Bourquin-Sherman[8] vitamin G-deficient diet, the vitamin potencies of which paralleled the intensities of yellow pigmentation.

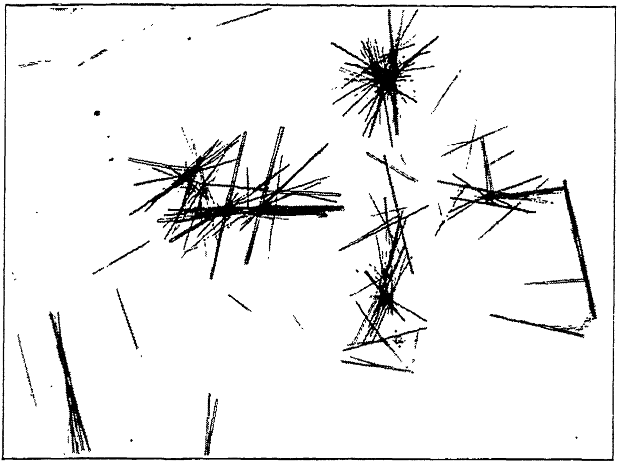

Fig. 1.—Riboflavin crystallized from water solution. Magnification 200 diameters.

Kuhn and his co-workers[9] had isolated a water-soluble yellow green-fluorescent pigment from dried egg albumin and reported[10] that the crystalline product was a growth-promoting supplement for the Bourquin-Sherman diet, 100 micrograms a day inducing a growth rate in rats of about 10 Gm. a week. In subsequent studies these workers seemingly had difficulty with the

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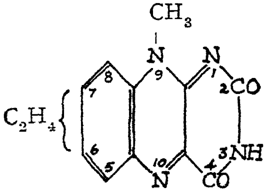
[7] Booher, Lela E.: The Concentration and Probable Chemical Nature of Vitamin G, J. Biol. Chem. **102:** 39-46 (Sept.) 1933.

[8] Bourquin, Anne, and Sherman, H. C.: Quantitative Determination of Vitamin G (B_2), J. Am. Chem. Soc. **53:** 3501-3505 (Sept.) 1931.

[9] Kuhn, Richard; György, Paul, and Wagner-Jauregg, Theodor: Ueber Ovoflavin, den Farbstoff des Eiklars, Ber. d. Deutschen chem. Gesellsch. **66:** 576-580 (April) 1933.

[10] Kuhn, Richard; György, Paul, and Wagner-Jauregg, Theodor: Ueber eine neue Klasse von Naturfarbstoffen (Vorläufige Mitteilung), Ber. d. Deutschen chem. Gesellsch. **66:** 317-320 (Feb.) 1933.

Bourquin-Sherman diet and found it necessary to supplement it further with concentrates of unidentified factors present in yeast extracts. These authors also called attention to the similarity in distribution of the vitamin and the water-soluble yellow pigments and to their probable relationship with the yellow enzyme. They suggested the chemical group name of flavins for these pigments, the ending "-in" denoting that they contained nitrogen as an elemental constituent. Since there appeared to be differences in the relative vitamin activities of the yellow pigment preparations, they assumed that there were inherent differences in chemical nature associated with variation in source materials. Pending further chemical investigations they proposed to differentiate them by appropriate prefixes such as ovo-, lacto- and hepato-flavins.



I. Lumiflavin—a predicted partial structural formula.

Further investigation has shown that an identical flavin having a d-ribose residue attached to the tricyclic chromophore nucleus (characteristic of all flavins) is obtained from a variety of natural source materials. Other flavins and flavin derivatives have been synthesized with various groups replacing the d-ribose residue and some of these possess vitamin activity. However, none of these now known possess greater vitamin potency than d-riboflavin and probably all of them are less potent than riboflavin.

The use of the term d-riboflavin or just riboflavin as a substitute for the terms vitamin B_2 or vitamin G was suggested[11] by the Council on Pharmacy and Chemistry. This terminology was later approved by the Committee on Vitamin Standards, American Society of Biological Chemists, and also by the Committee on

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[11] Council on Pharmacy and Chemistry: Riboflavin, the Accepted Name for Vitamin B_2, J. A. M. A. **108:** 1340-1341 (April 17) 1937.

Vitamin Nomenclature, American Institute of Nutrition (April 1937).

The remainder of the story of the chemistry of riboflavin is the story of the elucidation of the intimate structure of the molecule, its characteristic properties, its many derivatives and its relation to the "yellow oxidation enzyme."

ISOLATION

Riboflavin has been isolated from a wide variety of animal and plant products, including egg white,[9] milk,[12] liver,[13] kidney,[14] urine,[15] barley malt,[16] dandelion blossoms,[17] grasses,[18] egg yolk [19] and retinas of fish eyes.[20] It cannot be stated with absolute certainty that the crystalline flavin obtained from each of these various sources is chemically identical with riboflavin, but such is the case at least for those to which adequate determinative tests have been applied. Four synthetic flavins closely related chemically to riboflavin have been reported [21] to possess vitamin activity, but so far none of these have been reported to occur in natural products.

The isolation of riboflavin from these many naturally occurring source materials has been accomplished through the independent efforts of several laboratories. The several methods of isolation employed, although slightly diversified in certain detailed manipulations, have much in common. The successive steps in the isolation procedures may be summarized in a general way as follows: (1) extraction of flavin-containing material with aqueous acid solutions, with alcohol or with water-alcohol mixtures; (2) adsorption of the flavin from the extract on fullers' earth, lead sulfide or other suitable adsorbent usually from acid solution; (3) elution of the flavin from the adsorbent by means of pyridine, pyridine-methyl alcohol-water mixtures, or ammonia in water or in water-alcohol mixtures; (4) removal of elution solvent with recovery and concentration of the flavin in water or alcohol solution followed by elimination of acetone insoluble impurities; (5) precipitation and crystallization of a heavy metal (notably silver or thallium) salt of the flavin which is soluble with difficulty and easily crystallized, and (6) recovery of the metal-free flavin followed by repeated crystallizations from water, alcohol or dilute acetic acid solutions.

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[12] Kuhn, Richard; Rudy, Hermann, and Wagner-Jauregg, Theodor: Ueber Lactoflavin (Vitamin B$_2$) Ber. d. Deutschen chem. Gesellsch. **66:** 1950-1956 (Dec.) 1933.

[13] Karrer, Paul; Salomon, H., and Schöpp, K.: Isolierung des Hepaflavins, Helvet. chim. acta **17:** 419-425 (March) 1934.

[14] Karrer, Paul: Ueber einige natürlich vorkommende, biochemisch bemerkenswerte Pigmente, Helvet. chim. acta **19:** E 33-E 48 (Aug.) 1936.

[15] Koschara, Walter: Ueber ein Lyochrom aus Harn (Uro-flavin), Ber. d. Deutschen chem. Gesellsch. **67:** 761-766 (May) 1934. Karrer.[14]

[16] Karrer, Paul, and Schöpp, K.: Isolierung des Flavins aus Malz, Helvet. chim. acta **17:** 1013-1014 (Nov.) 1934.

[17] Karrer, Paul, and Schöpp, K.: Isolierung eines pflanzlichen Flavins, Helvet. chim. acta **17:** 771-772 (July) 1934.

[18] Kuhn, Richard, and Kaltschmitt, Hans: Isolierung von Lactoflavin (Vitamin B$_2$) aus Heu, Ber. d. Deutschen chem. Gesellsch. **68:** 128-131 (Jan.) 1935.

[19] Karrer, Paul, and Schöpp, K.: Isolierung des Lyochroms aus Eigelb (ovoflavin G), Helvet. chim. acta **17:** 735-737 (July) 1934.

[20] Euler, Hans, and Adler, Erich: Ueber das Vorkommen von Flavinen in tierischen Geweben, Ztschr. f. physiol. Chem. **223:** 105-112 (Feb.) 1934.

[21] Karrer, Paul, and Quibell, T. H.: Synthesen einiger neuer Flavine, Helvet. chim. acta **19:** 1034-1042 (Oct.) 1936.

In their earlier work, Kuhn and his co-workers [9] obtained from 100 Kg. of dried egg albumin, corresponding to about 33,000 eggs, 100 mg. of thrice recrystallized flavins. According to subsequent measurements [22] of the quantities of flavin normally present in dried egg albumin, this yield would correspond to around 7 per cent of the total flavin present in the egg albumin. Similarly the yield of crystalline flavin from milk as reported [23] in the earlier work was not greater than 5 per cent of the total quantity present. The use of heavy metal precipitations increased the yield to around 18 to 20 per cent of the quantity reported [22] to be normally present in milk.

Riboflavin crystallizes from absolute alcohol as yellow-orange, needle shaped crystal clusters. The crystals possess no sharp melting point but rather begin to darken at about 240 C. and decompose at temperatures of around 274 to 282 C. The tetraacetyl derivative liquefies with decomposition at about 238 to 242 C. Elementary analysis of the riboflavin crystals established the empirical formula $C_{17}H_{20}N_4O_6$ corresponding to a molecular weight of 376. A photomicrograph of riboflavin crystals (furnished through the courtesy of Dr. L. T. Work, Columbia University) deposited from a water solution is shown in Fig. 1.

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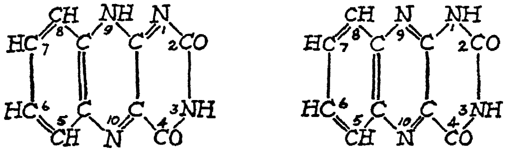
22. Kuhn, Richard; Wagner-Jauregg, Theodor, and Kaltschmitt, Hans: Ueber die Verbreitung der Flavine im Pflanzenreich, Ber. d. Deutschen chem. Gesellsch. **67**: 1452-1457 (Aug.) 1934.

23. Kuhn, Richard; György, Paul, and Wagner-Jauregg, Theodor: Ueber Lactoflavin, den Farbstoff der Molke, Ber. d. Deutschen chem. Gesellsch. **66**: 1034-1039 (July) 1933.

ELUCIDATION OF RIBOFLAVIN STRUCTURE

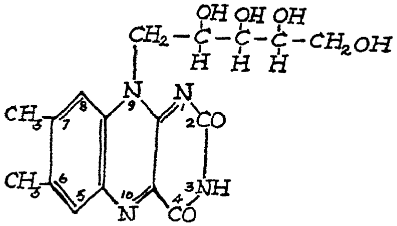
Following the isolation of riboflavin in crystalline form, the next step directed toward ultimate synthesis of the compound rested on elucidation of its characteristic chemical features. Warburg and Christian[4] had already isolated a photoderivative from the pigment component of their yellow enzyme and had expounded many of its more salient properties. Exposure of alkaline solutions of the pigment component to ultraviolet irradiation or ordinary light resulted in the formation of a yellow photoderivative (lumiflavin), soluble with difficulty in water but soluble in chloroform and having the empirical formula $C_{13}H_{12}N_4O_2$. The course of this reaction could be followed by acidifying a portion of the solution at intervals and extracting the solution with chloroform. This photoderivative exhibited spectral properties very similar to those of the pigment component of the enzyme having two characteristic absorption bands in the ultraviolet region and one in the visible wavelength range. The yellow color of the photoderivative could be reversibly dispelled by reduction with hydrogen in the presence of palladium. Warming a solution of the photoderivative in the presence of barium hydroxide liberated urea,[4] thereby disclosing its alkali lability and paving the way for the later disclosure of the presence of an -NH-CO-NH- grouping.



II. Iso-alloxazine and alloxazine.

The suggestion of a probable relationship of the yellow enzyme and the vitamin riboflavin stimulated no inconsiderable interest in this photoderivative. It was soon found[12] that irradiation of alkaline solutions of riboflavin resulted in the splitting off of a hydroxyl-rich side chain, the latter being responsible for the solubility of the vitamin in water. Since the elementary composition of both the vitamin ($C_{17}H_{20}N_4O_6$) and that of the photoderivative ($C_{13}H_{12}N_4O_2$) had been ascertained, the portion split off must correspond to $C_4H_8O_4$ ($C_{17}H_{20}N_4O_6 - C_{13}N_{12}N_4O_4 = C_4H_8O_4$). Furthermore, acetylation of riboflavin yielded a tetra-acetyl derivative associated with the 4- carbon portion split off by photolysis. One primary alcohol group in the hydroxyl-containing side chain was established from quantitative measurements of the formaldehyde formation resulting from the application of suitable oxidation procedures. The group split off then by photolysis must necessarly correspond to a side chain of the structure -CHOH.CHOH.CHOH.CH_2OH.

Kuhn and Rudy[24] made further intensive studies of the products of alkaline hydrolysis of riboflavin and lumiflavin. By heating alkaline solutions of either riboflavin or lumiflavin they obtained urea and an organic acid, $C_{12}H_{12}N_2O_3$, which contained one active hydrogen atom and which formed a bright yellow silver salt and gave a sharp monobasic acid titration curve. For the splitting off of urea by alkaline hydrolysis of lumiflavin, two molecules of water were required. This would indicate that the urea-yielding group belonged to a ring system and not to a side-chain ureide or guanidino group wherein only one molecule of water would be required. These observations, jointly considered, make it evident that both oxygen atoms of lumiflavin ($C_{13}H_{12}N_4O_2$) belong to an alkalilabile ring, one going to form urea and the other to build the carboxyl group in the organic acid $C_{12}H_{12}H_2O_3$.



III. d-riboflavin.

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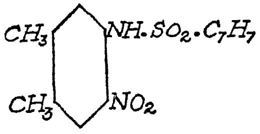
[24] Kuhn, Richard, and Rudy, Hermann: Ueber den alkali-labilen Ring des Lacto-flavins, Ber. d. Deutschen chem. Gesellsch. **67**: 892-898 (May) 1934.

Further studies[25] led to the finding that both lumiflavin and the organic acid $C_{12}H_{12}N_2O_3$ contained an alkylimide grouping, in contrast to riboflavin, in which this grouping is absent. Apparently then the sugar-like side-chain split off by photolysis of riboflavin was replaced by an alkyl group. As a result of the studies of the decomposition products of riboflavin and lumiflavin the partial structural formula I for the latter was advanced by Kuhn.

In the riboflavin molecule a d-ribityl group replaces the methyl group of lumiflavin at position 9. This proposed structure definitely related riboflavin and lumiflavin to the alloxazine series of organic compounds, which were first discovered by Kühling[26] in 1891 and which possessed many properties similar to those of flavins.

The structures of alloxazine and its tautomeric form, iso-alloxazine, are represented in formula II.



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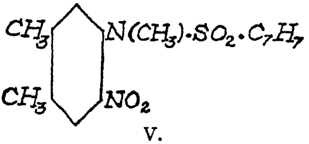

V.

Riboflavin and lumiflavin are derivatives of iso-alloxazine, since riboflavin and lumiflavin each possess only one active hydrogen atom, namely at position 3, while the alloxazine molecule contains two active hydrogen atoms (positions 1 and 3). In riboflavin and lumiflavin the hydrogen attached to the nitrogen at position 9 is replaced by a pentose residue and a methyl group, respectively.

A structural formula III was predicted for riboflavin and subsequently confirmed by synthesis. The formula shows the side-chain as ribose.

It was not known in the early stages of the investigations, of course, that the spacial relationships of the hydroxyl-containing side-chain corresponded specifically to d-ribose.

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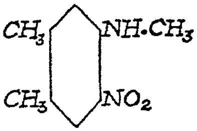
[25] Kuhn, Richard, and Rudy, Hermann: Ueber die Konstitution des Lumilactoflavins, Ber. d. Deutschen chem. Gesellsch. **67**: 1298-1299 (July) 1934.

[26] Kühling, O.: Ueber azine der Harnsäuregruppe, Ber. d. Deutschen chem. Gesellsch. **24**: 2363-2369 (July) 1891.

SYNTHESIS OF LUMIFLAVIN AND RIBOFLAVIN

Once the essential structural features of riboflavin had been deciphered, attempts to synthesize many of its chemically related compounds were quick to follow. The synthesis of lumiflavin was achieved before that of the vitamin. Except for the reaction involving condensation of the 6,7-dimethyl derivative of ortho-phenylenediamine with alloxan, a reaction based on the earlier work of Kühling [26] and Kühling and Kaselitz,[27] the steps involved in the synthesis of this compound are well known in the field of organic chemistry.

The synthesis of lumiflavin as developed by Kuhn and his co-workers [27a] is as follows: Ortho-xylene on treatment with nitric acid yields 1,2-dimethyl-4,5-dinitrobenzene, which on partial reduction yields 1,2-dimethyl-4-nitro-5-aminobenzene. The latter is heated at 100 C. with paratoluene sulfone chloride in pyridine for four to five hours and yields a product with formula IV. This intermediate on treatment with dimethyl sulfate and alkali at 50 to 60 C. yields a methyl derivative V. Hydrolysis of this derivative can be accomplished with a mixture of glacial acetic and concentrated sulfuric acids and there is produced the methyl amino compound VI. The nitro group of compound VI is reduced with stannous chloride and hydrochloric acid to yield the amino compound VII. The hydrochloride of compound VII is condensed with a slight excess of alloxan VIII in water at 50 to 60 C. to yield lumiflavin IX; the yield over all is about 75 per cent.



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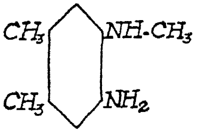

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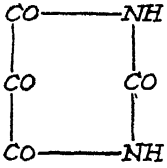

VIII.

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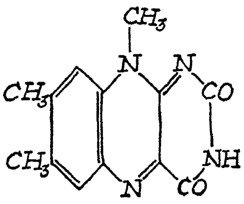
[27] Kühling, O., and Kaselitz, O.: Ueber Condensationsproducte N-substituirter O-Diamine mit Alloxan und dessen Derivaten, Ber. d. Deutschen chem. Gesellsch. **39**: 1314-1326 (March) 1906.

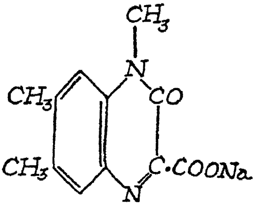
[27a] Kuhn, Richard, and Reinemund, Karl: Ueber die Synthese des 6, 7, 9, Trimethyl-flavins (Lumi-lactoflavins) Ber. d. Deutschen chem. Gesellsch. **67**: 1932-1936 (Nov.) 1934.

The alkaline hydrolysis of lumiflavin forms urea and an alkaline salt of the organic acid $C_{12}H_{12}N_2O_3$, X, a reaction of material assistance in elucidating the structure of the tricyclic chromophore nucleus of riboflavin.

Without the presence of the modified pentose side-chain attached to the N-atom position 9 (see proposed structural formula III for riboflavin) the flavin possesses no vitamin activity. Since the grouping -CHOH.-CHOH.CHOH.CH_2OH possesses three asymmetrical carbon atoms, there existed eight theoretically possible stereoisomers of the proposed structure, one of which presumably would be identical to that in natural riboflavin. The possibility was also tenable that more than one configuration would exhibit vitamin activity. The selection of possibilities which might yield a product identical with the naturally occurring vitamin could be somewhat narrowed in view of the optical activity of the vitamin as isolated. The vitamin exhibited optical activity only in alkaline solution, in which case it was levorotatory, and presumably the relative spacial relations of the -H and -OH groups nearest the chromophore group of the vitamin control the sign of rotation. This information narrowed the choice to l-arabinose, d-xylose and d-ribose residues for the hydroxyl-containing side-chain which might be identical with that in the vitamin molecule. The pentyl side-chain corresponding to d-ribose was finally proved to have the same spacial relationships as the side-chain involved in the naturally occurring vitamin. The synthetic d-riboflavin was identical in chemical properties with the vitamin as isolated from various source materials and



IX.

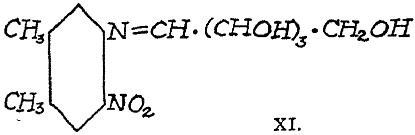


X. Salt of the acid $C_{12}H_{12}N_2O_3$.

the mixed melting point (attended with decomposition) of the synthetic and natural products indicated no depression of this constant.

The synthesis of d-riboflavin as developed by Kuhn and his co-workers [28] was the same as that for lumiflavin to the stage of formation of 1,2-dimethyl-4-nitro-5 amino-benzene. This compound on heating with d-ribose yields compound XI. The latter can be reduced with hydrogen and platinum black to give XII. On treatment with alloxan in a mixture of acetic and boric acids, a condensation occurs with the formation of d-riboflavin III.

Karrer and his co-workers [29] meanwhile had also developed a method of synthesis for d-riboflavin and recently [30] have described an improved method giving a yield of about 38 per cent of the theoretical yield calculated on the basis of the quantity of d-ribose employed. Starting with 2 Gm. of d-ribose they obtained approximately 2 Gm. of riboflavin.



XI.

The chief obstacle involved in the preparation of the synthetic vitamin riboflavin is the limited availability of the essential d-ribose. The supply of d-ribose used by both Karrer [30] and Kuhn [28] was prepared from d-arabinose, which in turn was obtained from the calcium salt of d-gluconic acid, a synthesis of five steps summarily attended with considerable losses. Kuhn and his co-workers [28] report that from 2 Kg. of the calcium salt of d-gluconic acid (yielding about 500 Gm. of d-arabinose) they obtained 53 Gm. of d-ribose.

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[28] Kuhn, Richard; Reinemund, Karl; Weygand, Friedrich, and Ströbele, Rudolf: Ueber die Synthese des Lactoflavins (Vitamin B_2). Ber. d. Deutschen chem. Gesellsch. **68**: 1765-1774 (Sept.) 1935.

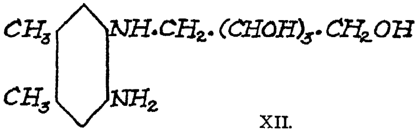
[29] Karrer, Paul; Becker, B.; Benz, F.; Frei, P.; Salomon, H., and Schöpp, K.: Zur Synthese des Lactoflavins, Helvet. chim. acta **18**: 1435-1448 (Oct.) 1935.

[30] Karrer, Paul, and Meerwein, H. F.: Eine verbesserte Synthese des Lactoflavins und 6, 7-dimethyl-9-[1'-arabityl]-iso-alloxazins, Helvet. chim. acta **19**: 264-269 (March) 1936.

The l-araboflavin has been synthesized[31] and found to possess vitamin activity, but in comparison to d-riboflavin it has been reported[32] to be only about one third as potent.

PROPERTIES OF RIBOFLAVIN AND RELATED COMPOUNDS

Syntheses of many derivatives of riboflavin have demonstrated the extremely limited alterations of the structural configuration of the riboflavin molecule compatible with vitamin activity. While the tricyclic chromophore nucleus is common to all flavins, the position and kind of substituent groups on the benzene ring and the nature of the side-chain attached to the nitrogen atom in position 9 are determinate factors for vitamin activity. The following synthetic flavins have been shown to possess vitamin activity: 6,7-dimethyl-9-[d,l'ribityl]-iso-alloxazine (riboflavin),[33] 6,7-dimethyl-9-[1,l'arabityl]-iso-alloxazine (araboflavin),[31a] 7-monomethyl-9-[d,l'ribityl]-iso-alloxazine,[21] 6-monomethyl-9-[d,l'ribityl]-iso-alloxazine[21] and 6 ethyl-7 methyl-9-[d,l'ribityl]-iso-alloxazine.[21]



XII.

At least one of the methyl groups in position 6 or 7 is essential in order that the flavin molecule shall possess vitamin activity. The absence of both the 6 and 7 methyl groups actually appears to be accompanied with toxicity.[34] Alterations of the riboflavin molecule by replacement of the methyl group at position 6 with one in position 5 or of the methyl group in position 7 with one in position 8 destroys the vitamin and

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31. (a) Kuhn, Richard, and Weygand, Friedrich: Synthetisches Vitamin B_2, Ber. d. Deutschen chem. Gesellsch. **67**: 2084-2085 (Dec.) 1934. (b) Karrer, Paul; Schöpp, K.; Benz, F., and Pfaehler, K.: Synthesen von Flavinen III, Helvet. chim. acta **18**: 69-79 (Dec.) 1935.

32. Kuhn, Richard; Rudy, Hermann, and Weygand, Friedrich: Ueber die Zuckerähnliche Seitenkette des Lactoflavins, Ber. d. Deutschen chem. Gesellsch. **68**: 625-634 (April) 1935.

33. Kuhn, Reinemund, Weygand and Ströbele.[28] Karrer and Meerwein.[30]

34. Kuhn, Richard, and Boulanger, Paul: Ueber die Giftigheit der Flavine, Ztschr. f. physiol. Chem. **241**: 233-238 (July) 1936.

co-enzyme activity.[34a] As regards the side-chain, only d-ribose or l-arabinose residues attached to the nitrogen atom in position 9 have thus far proved to be compatible with vitamin activity of the flavins. Exceedingly small variations in this side-chain are attended with complete lack of vitamin activity. Kuhn and Ströbele [34b] have recently synthesized 6, 7 dimethyl-9-d-ribosido-flavin wherein the d-ribose is attached to the tricyclic chromophore nucleus through a glucoside linkage. This flavin compound is totally devoid of vitamin and co-enzyme activity.

Fluorescence.—One of the most characteristic properties of riboflavin is its yellow green-fluorescence in neutral solutions. The fluorescence is at a maximum in the region of p_H 6.0 to p_H 7.0 and decreases in either more acid or more alkaline solutions. This observation would indicate that riboflavin was amphoteric in nature and that the electrically neutral molecule was responsible for the fluorescence. Kuhn and Moruzzi [35] made a series of fluorescence measurements of riboflavin solutions with graded p_H values. At p_H 1.7, on the acid side, and at p_H 10.2, on the alkaline side, the fluorescence was 50 per cent of its maximum value. Using these data in the generally accepted manner for calculation of the dissociation constants of base, acid and iso-electric point they obtained the following values:

$$k_{acid} = 63 \text{ X } 10^{-12},\ k_{base} = 0.5 \text{ X } 10^{-12} \text{ and}$$
$$\text{iso-electric point, } p_H \text{ 6.0.}$$

These authors used ultraviolet rays and were of the opinion that in the region of optimum p_H the fluorescence brightness was proportional to the riboflavin concentration.

Later, Karrer and his co-workers [36] reported that maximum fluorescence of solutions (p_H 7.0) of riboflavin appeared with concentrations of 0.003 per cent, the fluorescence intensity decreasing with either greater or lesser concentrations. They suggest that Kuhn and his co-workers probably used such dilute flavin solutions that the measurements were all made far below the maximum fluorescence brightness.

[34a] Kuhn, Richard; Desnuelle, Pierre, and Weygand, Friedrich: Zur Spezifität des Lactoflavins; die Bedeutung der Stellung der Methylgruppen, Ber. d. Deutschen. Chem. Gesellsch. **70:** 1293-1301 (June) 1937. Kuhn, Richard; Vetter, Hellmuth, and Rzeppa, H. W.: Zur Spezifität des Lactoflavins; Ersatz der Methylgruppen durch den Tetramethylen- und Trimethylen-Ring. Ber. d. Deutschen. Chem. Gesellsch. **70:** 1302-1314 (June) 1937.

[34b] Kuhn, Richard, and Ströbele, Rudolf: Synthese von Flavinglucosiden. Ber. d. Deutschen. Chem. Gesellsch. **70:** 747-752 (April) 1937.

[35] Kuhn, Richard, and Moruzzi, Giovanni: Ueber die Dissoziationskonstanten der Flavine; p_H-Abhängigkeit der Fluorescenz, Ber. d. Deutschen chem. Gesellsch. **67:** 888-891 (May) 1934.

[36] Karrer, Paul, and Fritzsche, H.: Fluorescenz-kurven des Lactoflavins und synthetischer Flavine, Helvet. chim. acta **18:** 911-914 (May) 1935.

The fluorescence measurements for lumiflavin gave practically the same k_{acid} and k_{base} constants as those for riboflavin, indicating that the sugar-like side-chain of riboflavin has very little influence on the electrolytic properties of the molecule.[35]

The retinas of the eyes of many species of animals have been reported[20] to contain relatively high concentrations of flavin, the concentration corresponding rather closely to that at which maximum fluorescence occurs. It is supposed that the flavins are involved in some balanced light sensitized reactions concerned with dim vision.

Optical Activity.—In neutral solutions or in acid solutions wherein the riboflavin exists as a positively charged ion, the optical activity of riboflavin is exceedingly small. In nonfluorescent alkaline solutions of riboflavin the maximum rotation (levo-) was observed[37] when one mole of riboflavin is in the presence of one mole of sodium hydroxide. This maximum rotation was given as $[\alpha]^{20}_{D} = \frac{-0.51^\circ \times 100}{0.447 \times 1} = -114^\circ$ (in 0.1 N NaOH). Karrer and Fritzsche[38] confirm this dependence of specific rotation on the relative concentrations of riboflavin and alkali.

Solubility and Stability.—Riboflavin, owing to the presence of its d-ribityl side-chain, is a typically water-soluble type of compound and is insoluble in the ordinary fat solvents. By splitting off this d-ribityl side-chain the resultant molecule becomes soluble in such solvents as chloroform.

Riboflavin is quite stable in strong mineral acids but sensitive to alkali. Riboflavin is sensitive to light and on irradiation with ultraviolet rays or visible light undergoes irreversible decomposition. Irradiation of alkaline solutions is attended with the formation of lumiflavin, $C_{13}H_{12}N_4O_2$. Irradiation of neutral or acid solutions of riboflavin is attended with the formation of 6,7-dimethyl-alloxazine, or "lumichrome,"[39] which exhibits an intense blue fluorescence.

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[37] Kuhn, Richard, and Rudy, Hermann: Ueber die optische Aktivität des Lacto-flavins, Ber. d. Deutschen chem. Gesellsch. **68**: 169-170 (Jan.) 1935.

[38] Karrer, Paul, and Fritzsche, H.: Die optische Aktivität des Lacto-flavins, Helvet. chim. acta **18**: 1026-1027 (June) 1935.

Riboflavin is quite stable to oxidizing agents, including hydrogen peroxide, bromine water and concentrated nitric acid. It is oxidized by chromic acid, yielding ammonia, carbon dioxide and a nitrogen-free residue.

Riboflavin is reversibly reduced by hydrogen (palladium or platinum and hydrogen, zinc in presence of acid), by sodium thiosulfate and by hydrogen sulfide in alkaline solution and by titanous chloride.

Oxidation-Reduction Potential—The very unique as well as complicated oxidation-reduction systems of the flavins have attracted unusual attention and also have caused a considerable volume of literature to be built up.

The flavins represent very stable oxidation-reduction systems, imparting quite reproducible potentials to noble metal electrodes within a reasonable range of buffering capacity. In relation to molecular structure it has been shown[40] that acetylation, methylation or loss of the sugar-like side-chain of riboflavin does not materially affect the oxidation-reduction potential of the flavin. Titration of the completely reduced flavin with a suitable oxidant (or vice versa) at graded p_H values forms the basis for a series of curves which have been variously reported as corresponding to a one electron system and a two electron system and an intermediate electron system. Apparently the electron number shifts and is different at different ranges of p_H values, which would account for some of the divergence in reports. According to Stern,[41] the slope of the titration curves in the range of p_H 6.0 to 12.4 corresponds to that of a two electron system. From p_H 4.0 to p_H 1.0 the character of the curves is that of a one electron system, while at p_H values of less than one the steepness of the curves gradually increases until at p_H 0.4 two maxima appear, indicating a two-stage process. At p_H values less than 5.0 a red intermediate compound, as first noted by Kuhn and his co-workers,[42] appears, which becomes more pronounced as the two-stage separation is approached at about p_H 0.4.

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[39] Karrer, Paul; Salomon, H.; Schöpp, K.; Schlittler, E., and Fritzsche, H.: Ein neues Bestrahlungs-produkt des Lactoflavins: Lumichrom, Helvet. chim. acta **17:** 1010-1013 (Oct.) 1934.

[40] Kuhn, Richard, and Moruzzi, Giovanni: Ueber das Reduktions-Oxydations-Potential des Lacto-flavins und seiner Derivate, Ber. d. Deutschen chem. Gesellsch. **67:** 1220-1223 (July) 1934.

[41] Stern, K. G.: Potentiometric Study of Photo-flavins, Biochem. J. **28:** 949-964 (March) 1934.

The normal potential of riboflavin referred to the normal hydrogen electrode is in the region of —0.21V

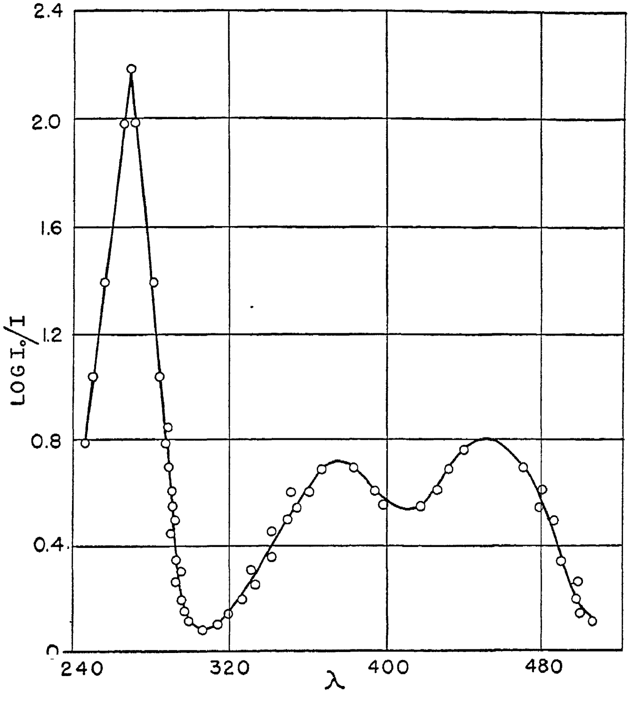

Fig. 2.—Absorption characteristics of riboflavin.
Maximum molecular extinction coefficient.

$$\frac{2.30}{\text{x d}} \text{ x log } \frac{}{\text{I}}$$

c = 0.0025 per cent, d = 1.0 cm.
κ at 267 μ = 7.6 x 10^4.

(p_H 7.0) as reported by many investigators, including those referred to. The physiologic significance of the extremely negative position of the normal potentials of the flavins is not at present understood. Under the general anaerobic reduction potential of living cells, the cell flavin is said [43] to be exactly in its equilibrium range.

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[42] Kuhn, Richard, and Wagner-Jauregg, Theodor: Ueber das Reduktions-Oxydations-Verhalten und eine Farbreaktion des Lacto-flavins (Vitamin B_2), Ber. d. Deutschen chem. Gesellsch. **67**: 361-363 (Feb.) 1934.

In a recent report, Kuhn and Ströbele [44] state that in the conversion of riboflavin to leuko-riboflavin no less than three different reduction intermediates intervene and that these are distinguishable by vivid colors and sharp definite compositions.

Absorption of Light by Flavins.—Riboflavin, lumiflavin and the yellow enzyme exhibit characteristic absorption bands with well defined maxima in approximately the same wavelength regions. It is apparent, therefore, that this property resides in the tricyclic chromophore nucleus of the flavins. Riboflavin exhibits definite maxima at approximately 220, 267, 366 and 446 millimicrons. The green fluorescence extends from about 500 to 630 millimicrons. The use of the extinction coefficient especially in connection with the wavelength maximum at 267 millimicrons is useful in measuring the relative purity of riboflavin preparations.

The absorption characteristics of riboflavin in water solution are shown [44a] graphically in Fig. 2.

Flavin Combination in Natural Products.—At least 90 per cent of the riboflavin in milk is in a free dialysable form. In most other materials, such as liver, yeast and green leaves, it occurs in combination with compounds of high molecular weight.[45]

VITAMIN ACTIVITY OF RIBOFLAVIN

No fewer than a dozen reports have appeared which carried some semblance of a quantitative relationship between riboflavin dosage and the growth rate of rats on a riboflavin-deficient diet. In many cases, however, such meager data were supplied in these reports relative to the control of the experimental animals that critical examination of the work is impossible. It would seem probable that no two groups of workers had used the same riboflavin-deficient diet and probably most of them had not completely eliminated the practice of coprophagy in the experimental animals. The action of micro-organisms is responsible for the formation of flavin in the lower colon.

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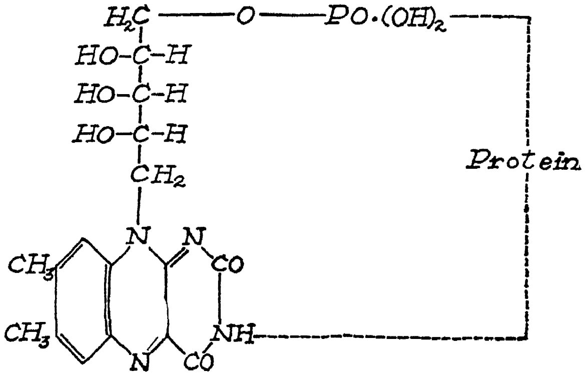
[43] Stern, K. G.: Uroflavin, Maltoflavin and Redox-Potentials of Lyochromes, Nature **133:** 178-179 (Feb. 3) 1934.

[44] Kuhn, Richard, and Ströbele, Rudolf: Ueber Verdo-, Chloro- und Rhodoflavine, Ber. d. Deutschen chem. Gesellsch. **70:** 753-760 (April) 1937.

[44a] I wish to thank Mrs. G. H. Haupt, National Bureau of Standards, for taking the transmittancy measurements of solutions of riboflavin used in preparation of this figure.

[45] Kuhn, Richard, and Kaltschmitt, Hans: Ueber den Zustand des Vitamins B_2 in der Kuhmilch, Ber. d. Deutschen chem. Gesellsch. **68:** 386-387 (Feb.) 1935.

Kuhn and his co-workers[12] have reported that 7 micrograms of riboflavin was equivalent to a Bourquin-Sherman unit when the Bourquin-Sherman Vitamin G-deficient diet[8] was supplemented with vitamin B_4. In other words, they state than 7 micrograms of pure riboflavin will support a growth rate of 3 Gm. a week in standardized rats receiving this modified Bourquin-Sherman diet.



XIII. Yellow oxidation enzyme (Warburg).

Von Euler and his co-workers,[46] using a somewhat different diet, report that 2 micrograms of riboflavin correspond to a Bourquin-Sherman unit.

In my experience (unpublished data), 3 micrograms of crystalline riboflavin per rat daily as a supplement to the Bourquin-Sherman diet regularly induced a weekly growth rate of 3.1 ± 1.0 Gm. The practice of coprophagy in these experiments was completely eliminated and the vitamin G-deficient diet was prepared as originally described by Bourquin and Sherman.

The riboflavin requirements are closely related to body size or more particularly to mass of active tissue, a further fact which may help to explain some of the divergence in vitamin values reported for riboflavin.

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[46] von Euler, H.; Karrer, Paul; Adler, E., and Malmberg, M.: Ueber die Wachstumswirkung der Flavine, Helvet. chim. acta **17**: 1157-1165 (Oct.) 1934.

RELATION OF RIBOFLAVIN TO THE YELLOW ENZYME

The yellow oxidation enzyme discovered by Warburg and Christian has been isolated in crystalline form by Theorell.[47] This isolation was accomplished by a combination of cataphoresis and fractionation with ammonium sulfate. Cataphoresis was used principally to separate the enzyme from large amounts of polysaccharides, which would interfere with the subsequent fractionation.

The reversible splitting of the protein and riboflavin components of this enzyme was accomplished by dialysis against dilute hydrochloric acid at 0° followed by dialysis against water. By mixing the electrolyte-free water solutions of the components in the cold, the activity of the enzyme for the most part returned.[47]

The molecular weight of the enzyme as calculated by Kerwick and Pedersen [48] from the sedimentation and diffusion data, sedimentation equilibrium data and assuming that the enzyme contains one molecule of riboflavin (molecular weight 376) per molecule of enzyme, gave values in the neighborhood of 80,000. The iso-electric point of the enzyme was found to be about p_H 5.2.

Theorell [49] furthermore found that riboflavin existed in the enzyme as a phosphoric acid ester, a nucleotide combination in which the purine is replaced by riboflavin. Kuhn and his co-workers [50] have succeeded in synthesizing the riboflavin-5′-phosphoric acid ester (6,7-dimethyl-9-d-riboflavin-5′-phosphoric acid) and find it identical with cytoflav from heart muscle. The phosphoric acid radical is, in fact, one of the connecting links between the riboflavin and the protein component of the enyzme. It has been postulated [51] that the protein component of the enzyme is bound to the phosphoric acid radical and to the free imide group in position 3 of the riboflavin-5′-phosphoric acid ester. Riboflavin itself is much less effective than its phosphoric acid ester in combining with the protein component, while a blocking of the hydrogen atom of the imide group in position 3 prevents the combination of protein and flavin components. Kuhn and his co-workers have, as a result of these observations, expressed the combination of protein and riboflavin phosphoric acid ester as shown in formula XIII. A less active enzyme is formed by combination of riboflavin (unesterified) and the protein component solely through the imide grouping of the former at position 3. This combination is broken by dialysis against distilled water at p_H 7.0.

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[47] Theorell, Hugo: Reindarstellung (Kristallisation) des Gelben Atmungsfermentes und die reversible Spaltung desselben, Biochem. Ztschr. **272:** 155-156 (July) 1934.

[48] Kerwick, R. A., and Pedersen, K. O.: Some Physicochemical Characteristics of the Yellow Respiratory Enzyme, Biochem. J. **30:** 2201-2205 (Dec.) 1936.

[49] Theorell, Hugo: Ueber die Wirkungsgruppe des Gelben Ferments, Biochem. Ztschr. **275:** 37 (Dec.) 1934.

[50] Kuhn, Richard; Rudy, Hermann, and Weygand, Friedrich: Ueber die Bildung eines kunstlichen Ferments aus 6, 7-dimethyl-9-1-araboflavin-5′-phosphorsäure, Ber. d. Deutschen chem. Gesellsch. **69:** 2034-2036 (Sept.) 1936.

[51] Kuhn, Richard, and Rudy, Hermann: Katalytische Wirkung der Lactoflavin-5′-phosphorsäure, synthese des Gelben Ferments, Ber. d. Deutschen chem. Gesellsch. **69:** 1974-1977 (Aug.) 1936.

The yellow oxidation enzyme contains 15.9 per cent of nitrogen and 0.043 per cent of phosphorus. It also possesses at least three distinct absorption maxima at wavelengths of 265, 380 and 465 millimicrons. Recently Weygand and Stocker[51a] have developed a relatively large-scale process for the isolation of the yellow ferment from yeast. Repeated adsorption of the dialyzed enzyme solution on aluminum hydroxide suspensions (Willstätter's ortho-aluminum hydroxide γ), elution with solutions of ammonia and diammonium phosphate in certain proportions and repeated precipitations with ammonium sulphate constituted the main steps in this process. The yields of riboflavin phosphoric acid contained in a given weight of dry yellow enzyme indicated a molecular weight of the protein fraction of 74,000. The development of this large-scale process made it possible for Kuhn and Desnuelle[51b] to avail themselves of sufficient quantities of the yellow enzyme for studies of the amino acid composition of the protein fraction of the yellow enzyme. Arginine, histidine, and lysine accounted for 24.7 per cent of the weight of the protein fraction. The yields of tyrosine, cystine, phenylalanine, tryptophane and glutamic acid together accounted for 25.8 per cent of the protein fraction. In all they were able to account for about 65 per cent of the total nitrogen of the protein fraction of the enzyme.

[51a] Weygand, Friedrich, and Stocker, Hans: Reindarstellung von gelbem Ferment aus Hefe durch Adsorptionsverfahren, Ztschr. f. Physiol. Chem. **247**: 167-171 (June) 1937.

[51b] Kuhn, Richard, and Desnuelle, Pierre: Uber die Aminosäuren des gelben Ferments, Ber. d. Deutschen. Chem. Gesellsch. **70**: 1907-1926 (Sept.) 1937.

For further details of the chemical and enzymatic properties of this enzyme the reader is referred to the review by Theorell.[52]

The rôle of riboflavin as a component of an oxidation enzyme present presumably in all living cells explains some of the observations which were so baffling only a few years ago. Animals subsisting on riboflavin-deficient diets for any considerable period were invariably in a more critical condition than outward and obvious signs could indicate. They appeared for quite some time before death to be on the verge of collapse. This condition would seem to be a necessary corollary to the cellular asphyxiation brought about by a lack of a cellular oxidation catalyst.

SUMMARY

Riboflavin (6,7-dimethyl-9- [d-1′ribityl] -iso-alloxazine) is a yellow green-fluorescent water-soluble pigment widely distributed throughout both the plant and the animal kingdoms.

Through its iso-alloxazine nucelus it is related to and possesses a combination of the chemical properties of a substituted benzene, an azine dye and a pyrimidine. In addition, riboflavin possesses a ribityl side-chain attached to the N-atom in position 9, which relates it to the pentose sugars.

Riboflavin forms a phosphoric acid ester (riboflavin-5′-phosphoric acid) which combines with a protein to yield a yellow oxidation enzyme. This enzyme is presumably present in every living cell, at least in the cells of all higher forms of life, and is concerned with the chemical reactions involved in cell respiration.

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[52] Theorell, Hugo: Das Gelbe Oxidationsferment, Biochem. Ztschr. **278**: 263-290 (June) 1935.

CHAPTER XIV

RIBOFLAVIN

PHYSIOLOGY AND PATHOLOGY

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COLUMBIA, MO.

In the course of their studies on pellagra Goldberger and Lillie[1] used rats as experimental animals, and produced a characteristic deficiency disease. Ophthalmia and bilaterally symmetrical denuded areas were the most important symptoms, and these are still the most common and most characteristic. Goldberger and Lillie reported that "some of the animals have developed a dermatitis at one or more of the following sites: ears, front of neck and upper part of chest, forearms, backs of forepaws, shins, and the backs of the hind paws." Other workers[2] also observed the more severe symptoms but they are comparatively infrequent. Goldberger's description was reviewed by Sure,[3] so additional details will be omitted. The agent that prevents the lesions was designated by Goldberger as the P-P (pellagra-preventive) factor. American biochemists designated the new vitamin as G, the British workers called it vitamin B_2. Goldberger made a very important contribution, but, as is now known, most of the symptoms of the rat disease he described are prevented by riboflavin. This substance does not prevent pellagra, so the two diseases are not analogous.

Goldberger and Lillie did not report the incidence of the symptoms due to a lack of their P-P factor, but other workers have encountered the most extreme variability. Some workers report no symptoms except failure to grow. Others produce them readily in one trial and have difficulty in another.[4] Roscoe [4] reported that of 191 rats deprived of vitamin B_2, a little less than 57 per cent developed dermatitis in an average period of ten weeks. For some years these inconsistencies were puzzling, and it was not possible to eliminate them until the technic was further improved. Hogan and Hunter [5] had announced that the new factor vitamin G is destroyed by ultraviolet irradiation. It eventually developed that this irradiation not only destroyed vitamin G (or riboflavin in the more recent terminology) but also destroyed another vitamin which had not been recognized at that time. Since the symptoms of these two deficiencies sometimes have a superficial resemblance, neither could be sharply defined until both were more or less fully understood.

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[1] Goldberger, Joseph, and Lillie, R. D.: A Note on an Experimental Pellagra-like Condition in the Albino Rat, Pub. Health Rep. **41:** 1025 (May 28) 1926.

[2] Richardson, L. R., and Hogan, A. G.: Skin Lesions of the Rat Associated with the Vitamin B Complex, Research Bull. 241, Missouri Agric. Exper. Sta., 1936.

[3] Sure, Barnett: The Present Status of Vitamin B_2 (G), J. A. M. A. **99:** 26 (July 2) 1932.

The technic of Hogan and Hunter was modified [6] in certain respects, and when the modified procedure was used a characteristic dermatitis developed in every experimental animal that survived the depletion period. These lesions have only a superficial resemblance to those described by the previously mentioned workers, and since they did not appear when the rations contained corn starch it was suggested that there are several types of dermatitis, each due to a different deficiency. This suggestion was soon corroborated.[7] The experimental procedure not only demonstrated that there are two different types of lesions, each the result of a different deficiency disease, but it made it possible to produce either, consistently, and with an incidence of practically 100 per cent. It has been suggested[8] that in conformity with the American system of nomenclature the new vitamin be designated as H. It is B_6 in the British terminology. Since the term vitamin G as first used included at least two vitamins, there is no agreement as to what its precise meaning should be now. Evidence obtained with the rat shows that the old vitamin B complex contains vitamin B (B_1), riboflavin and H (B_6); there is no doubt that at least one more must be added[2] and several more have been suggested.

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[4] Chick, Harriette, and Roscoe, Margaret H.: Dual Nature of Water-Soluble Vitamin B.: II. Effect upon Young Rats of Vitamin B_2 Deficiency and a Method for the Biological Assay of Vitamin B_2, Biochem. J. **22**: 790, 1928. Roscoe, Margaret H.: Note on the Incidence of Dermatitis Among Rats Deprived of Vitamin B_2, Biochem. J. **27**: 1533 (No. 5) 1933.

[5] Hogan, A. G., and Hunter, J. E.: The Plural Nature of Vitamin B, J. Biol. Chem. **78**: 433 (July) 1928.

[6] Hogan, A. G., and Richardson, L. R.: The Effect of Ultra-Violet Rays on the Dermatitis Preventing Vitamin, Research Bull. 178, Missouri Agric. Exper. Sta., 1932.

[7] Hogan, A. G., and Richardson, L. R.: Effect of Ultraviolet Irradia-

Rats, Nature **133**: 498, 1934. Chick, Harriette; Copping, Alice M., and Edgar, Constance E.: The Water-Soluble B-Vitamins: IV. The Components of Vitamin B_2, Biochem. J. **29**: 722 (No. 3) 1935. Harris, L. J.: Flavin and the Pellagra-Preventing Factor as Separate Constituents of a Complex Vitamin B_2, ibid. **29**: 776 (No. 3) 1935. Halliday, Nellie, and Evans, H. M.: Dietary Production of the Syndrome of Deficiency in Vitamin B_6, J. Nutrition **13**: 657 (June) 1937. Booher, Lela E.: The Concentration and Properties of Vitamin H, J. Biol. Chem. **119**: 223 (June) 1937.

The ration of Richardson and Hogan proved to be well adapted also for studies on riboflavin deficiencies. Unpublished data show that 155 rats were on rations deficient in this substance long enough for the characteristic denuding symptoms to develop. Of these, 136, or 95 per cent, became denuded in periods ranging from six to twenty-four weeks. Some of the remaining 5 per cent were used for other purposes during that period; so with the technic now available practically every rat that survives long enough will develop the typical symptoms.

IDENTITY OF VITAMIN G (B_2) AND RIBOFLAVIN

To digress for a moment, Blyth[9] had demonstrated the presence in milk of a pigment which he called lactochrome. In more recent years several groups of workers became interested in this substance, and more or less concentrated preparations were obtained[10] from milk, liver, heart, kidney, muscles, egg white and yeast. Since the pigment was first isolated in crystalline form[11] from milk, it was designated as lactoflavin, but it is more commonly known as riboflavin in the United States. This substance is yellow, with a characteristic green fluorescence.

Kuhn and his collaborators were interested in vitamin G (B_2), and early in their work they observed a parallelism between vitamin B_2 activity and the intensity of the yellow color and green fluorescence. Both the vitamin and the pigment were destroyed on exposure to light, so it was assumed that they were identical, and this assumption was confirmed.[12] Of the various animal tissues examined, liver and kidney contain the largest amount. One unit is contained in from 0.2 to 0.4 Gm. of fresh weight of liver and kidney and in 0.5 Gm. of ox heart. The heart muscle contains about five times as much of the vitamin as does striated muscle. Their unit is from 8 to 10 micrograms of riboflavin, the amount that permits a gain by a rat of 40 Gm. in thirty days. Edgar and her collaborators[13] report that approximately 40 micrograms daily is required for optimum growth.

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[8] Richardson and Hogan.[2] Booher.[7]

[9] Blyth, A. W.: The Composition of Cow's Milk in Health and Disease, J. Chem. Soc., London **35**: 530, 1879.

[10] Ellinger, P., and Koschara, W.: Ueber eine neue Gruppe tierischer Farbstoffe (Lyochrome), Ber. d. deutschen chem. Gesellsch. **66**: 315, 1933. Kuhn, Richard; György, Paul, and Wagner-Jauregg, Theodor: Ueber eine neue Klasse von Natur-farbstoffen, ibid. **66**: 317, 1933.

[11] Kuhn, Richard; György, Paul, and Wagner-Jauregg, Theodor: Ueber Lactoflavin, den Farbstoff der Molke, Ber. d. deutschen chem. Gesellsch. **66**: 1034, 1933.

György and his collaborators observed that if liver or yeast is extracted in the cold a small portion of vitamin B_2 will dialyze, but that if the extracting fluid is heated then it all dialyzes readily. Evidently some of the vitamin is free and the remainder is bound to a carrier of high molecular weight. This is also true of riboflavin: some is free and some is bound to a colloidal carrier, so the pigment is probably both an enzyme and a vitamin. When the union is broken the enzymic properties are lost, but the vitamin effect remains.

Karrer, Salomon and Schöpp[14] determined the free flavin in liver; also the amount that is combined. One determination showed 76 per cent, another 53.5 per cent. in high molecular weight combination. In cow's milk about 90 per cent of the total amount is free, but Ellinger and Koschara[15] reported that in human milk the pigment is attached to a colloidal carrier, probably albumin.

More recently Karrer[16] and Kuhn[17] almost simultaneously accomplished the synthesis of riboflavin, and György[18] showed that the biologic activity of the synthetic product is identical with that of the naturally occurring form. Its activity is the same, whether administered orally or intraperitoneally.[19]

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[12] György, Paul; Kuhn, Richard, and Wagner-Jauregg, Theodor: Das Vitamin B_2, Naturwissenschaften **21:** 560, 1933.

[13] Edgar, Constance E.; Macrae, T. F., and Vivanco, Franciso: The Water-Soluble B-Vitamins: VII. Growth-Promoting Properties of Lactoflavin, Biochem. J. **31:** 879 (June) 1937.

[14] Karrer, P.; Salomon, H., and Schöpp, K.: Isolierung des Hepaflavins, Helvet. chim. acta **17:** 419, 1934.

[15] Ellinger, P., and Koschara, W.: The Lyochromes: A New Group of Animal Pigments, Nature **133:** 553, 1934.

[16] Karrer, P.; Schöpp, K., and Benz, F.: Synthesen von Flavinen: IV, Helvet. chim. acta **18:** 426, 1935.

[17] Kuhn, Richard; Reinemund, K.; Weygand, F., and Ströbele, R.: Ueber die Synthese des Lactoflavins (Vitamin B_2), Ber. d. deutschen chem. Gesellsch. **68:** 1765, 1935.

[18] György, Paul: Ueber die Wachstumswirkung synthetischer Flavinpräparate, Ztschr. f. Vitaminforsch. **4:** 223 (July) 1935.

RIBOFLAVIN AND CELL RESPIRATION

Various physiologic rôles have been ascribed to riboflavin, but the only one concerning which there is no dispute is that it has some function in the oxidation processes of the cell. Our knowledge of these reactions is chiefly due to Warburg and Christian. It is impossible in this paper to give an adequate review of their researches, but they[20] isolated a yellow oxidation enzyme from bottom yeast, which was of a new type, as shown by the fact that it is not inhibited by carbon monoxide or by hydrocyanic acid. The active group of the enzyme, the pigment portion, is bound to protein and is easily detached.[21] This protein-free pigment is yellow, with a green fluorescence, and according to Warburg and Christian it has no enzymic activity.

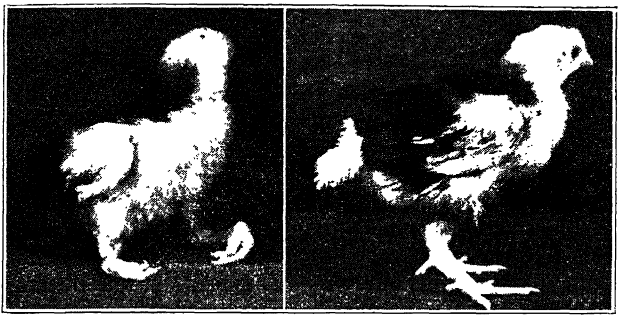

Fig. 1.—Although riboflavin deficiency is only now becoming recognized in man, it has appeared in animals. Photographs show the deficiency in Ancona chicks on a practical ration. The chicken in the figure to the left is 16 days old. The same bird is shown in the figure to the right 4 days later after receiving a daily supplement of 100 micrograms of crystalline riboflavin. Other chicks which received no riboflavin failed to recover.

In studies of the properties of the yellow enzyme, Robison's hexose-monophosphate ester was used as the substrate. It developed though that in the oxidation of the ester two other catalytic agents are required. These are a second enzyme (dehydrogenase or intermediary enzyme) which is colorless, and a coenzyme. The reactions of this type of respiration have been summarized[22] as follows:

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[19] György, Paul: Growth-Promoting Activity of Lactoflavin Administered Orally and Parenterally, Proc. Soc. Exper. Biol. & Med. **35**: 207 (Oct.) 1936.

[20] Warburg, Otto, and Christian, Walter: Ueber ein neues Oxydationsferment und sein Absorptionsspektrum, Biochem. Ztschr. **254**: 438 (Oct. 24) 1932.

[21] Warburg, Otto, and Christian, Walter: Ueber das neue Oxydationsferment, Naturwissenschaften **20**: 980, 1932.

Coenzyme + R.CHO + H_2O → coenzyme H_2 + R.COOH
Coenzyme H_2 + yellow enzyme → coenzyme + yellow enzyme H_2
Yellow enzyme H_2 + O_2 → yellow enzyme + H_2O_2

The entire system, then, that oxidizes hexosephosphoric acid to phosphohexonic acid consists of a dehydrogenase (or intermediary enzyme), a coenzyme, the yellow oxidation enzyme and molecular oxygen.

Important details additional to our knowledge of the yellow oxidation enzyme were added by Theorell.[23] The enzyme was purified and found to have a nitrogen content of 15.5 per cent. A salt-free solution was then dialyzed against dilute hydrochloric acid and thus broken into two component parts, a protein and a fluorescent pigment, neither of which was able to transfer oxygen. They could be recombined, however, and the combination exerted normal enzymic activity. This protein fraction is specific and cannot be replaced by other native proteins such as globin. Theorell succeeded in isolating the prosthetic group of Warburg's yellow enzyme, which was identified as flavin phosphate. That the prosthetic group of yellow enzyme is a phosphoric acid ester is of considerable physiologic significance. It is true that free riboflavin may combine with the colloidal carrier to form an enzyme,[24] but this is probably of little quantitative significance.

A somewhat different approach to the physiology of riboflavin is due to the observations of Banga and Szent-Györgyi.[25] They used the heart muscle of swine and prepared a concentrate of "respiration-coferment." This concentrate also contained a golden yellow pigment, which they named "cytoflav." The absorption spectrum[26] of cytoflav showed that it has some structural similarity to Warburg's yellow enzyme, and it was shown by Kuhn and Rudy[27] that cytoflav is the phosphoric acid ester of riboflavin. It is significant that a glycerol extract of the small intestine of rats in 0.01 molar phosphate solution will convert flavin quantitatively to the phosphoric acid ester.[28]

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[22] Warburg, Otto; Christian, Walter, and Griese, Alfred: Wasserstoffübertragendes Co-Ferment, seine Zusammensetzung und Wirkungsweise, Biochem. Ztschr. **282:** 157 (Nov. 21) 1935.

[23] Theorell, Hugo: Reindarstellung (Kristallisation) des gelben Atmungsfermentes und die reversible Spaltung desselben, Biochem. Ztschr. **272:** [illegible] (July 23) 1934; Reindarstellung der Wirkungsgruppe des gelben Ferments, ibid. **275:** 344 (Jan. 5) 1935.

[24] Kuhn, Richard, and Rudy, Hermann: Lactoflavin als Co-Ferment; Wirkstoff und Träger, Ber. d. deutschen chem. Gesellsch. **69:** 2557, 1936.

[25] Banga, I., and Szent-Györgyi, A.: Ueber Co-Fermente, Wasserstoffdonatoren und Arsenvergiftung der Zellatmung, Biochem. Ztschr. **246:** 203 (March 15) 1932.

Presumably the necessity for including riboflavin in the diet is that it is an essential constituent of the yellow oxidation enzyme that cannot be synthesized by the animal cell. It is probable, then, that the vitamin activity of flavin is due to the fact that it forms an ester with phosphoric acid, and this ester combines with protein to form the yellow oxidation enzyme. Apparently, if no hemin substances are present, all of the cell respiration is accomplished by Warburg's yellow ferment. On the other hand, if iron compounds are present it may be that only a small part of cell respiration is due to this enzyme.

RIBOFLAVIN IN TUMOR TISSUE

As would be expected, the respiration of tumor cells has been given intensive study and a low concentration of vitamin B_2 (riboflavin) in carcinoma tissues has been reported.[29] Von Euler and Adler[29] found from 0.5 to 1 microgram of riboflavin per gram of fresh weight in the Roux sarcoma (chick) and from 0.025 to 0.5 microgram in the Jensen sarcoma (rat). Elliott, Benoy and Baker[30] studied two types of tumor tissue, the Philadelphia No. 1 rat sarcoma and the Walker No. 256 carcinoma. Of the various oxidative mechanisms studied, Elliott and his associates concluded that two and possibly three are not active in tumor tissue. They suggest that the low concentration of flavin may

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26. Laki, K.: Ueber Cytoflav, Biochem. Ztschr. **266:** 202 (Oct. 24) 1933.

27. Kuhn, Richard, and Rudy, Hermann: Wachstumswirkung von Flavinphosphorsäuren, Ztschr. f. physiol. Chem. **239:** 47 (Feb. 28) 1936.

28. Rudy, Hermann: Enzymatische Phosphorylierung des Lactoflavins, Naturwissenschaften **23:** 286, 1935.

29. György, Paul; Kuhn, Richard, and Wagner-Jauregg, Theodor: Verbreitung des Vitamins B_2 im Tierkörper, Ztschr. f. physiol. Chem. **223:** 21 (Feb. 5) 1934. von Euler, Hans, and Adler, Erich: Ueber das Vorkommen von Flavinen in tierischen Geweben, Ztschr. f. physiol. Chem. **223:** 105 (March 6) 1934.

30. Elliott, K. A. C.; Benoy, Marjorie P., and Baker, Zelma: The Metabolism of Lactic and Pyruvic Acids in Normal and Tumour Tissues: II. Rat Kidney and Transplantable Tumours, Biochem. J. **29:** 1937 (Aug.) 1935.

explain the failure of tumors to oxidize lactate or malate, though lack of enzymes or coenzymes would explain the failures equally well.

STORAGE AND EXCRETION OF RIBOFLAVIN

It has been reported[31] that the flavin content of organs cannot be much increased; even a tenfold increase in the amount of riboflavin consumed increased very slightly the amount of the yellow enzyme in the liver. On the other hand, the body guards its store of this substance and even in rats that die for lack of riboflavin the amount in the liver, kidney and heart is still about one third of the normal level.[32] When flavin was added to the diet of depleted animals, growth started before the reserves in the organs were restored to the original amount.

When the diet is adequate riboflavin is a normal excretory product, and Koschara[33] isolated crystalline uroflavin from urine. The typical yellow color of urine, however, is due to a different compound. According to Vivanco[32] the total excretion of flavin in the urine and feces of a rat is from 3 to 5 micrograms daily, and the feces contain about twice the amount present in urine. Within fourteen days after total deprivation of vitamin B_2 no flavin was excreted in the urine and growth was suspended.

The most extensive study of riboflavin excretion by man was conducted by Emmerie,[34] who reported that the daily elimination in the urine of males is from 819 to 1,250 micrograms or from 30 to 50 micrograms per hour. However, if the consumption of riboflavin is greatly increased there is a corresponding increase in the amount eliminated. In a continuation of this investigation, Emmerie[35] states that the body seems to act as a reservoir for flavin. On a very low intake the excretion exceeds the intake and on a high intake the excretion lags behind. After thirteen days of flavin restriction the urinary excretion varied between 43 and

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31. Kuhn, Richard; Kaltschmitt, Hans, and Wagner-Jauregg, Theodor: Ueber den Flavingehalt der Leber und Muskulatur von gesunden und B_2-avitaminotischen Ratten, Ztschr. f. physiol. Chem. **232:** 36 (March 11) 1935.

32. Vivanco, Francisco: Zur Flavinbilanz im Tierkörper, Naturwissenschaften **23:** 306. 1935. Kuhn, Kaltschmitt and Wagner-Jauregg.[31]

33. Koschara, W.: Ueber Harnlyochrome, Ztschr. f. physiol. Chem. **232:** 101 (March 25) 1935.

34. Emmerie, A.: Determination and Excretion of Flavins in Normal Human Urine, Nature **138:** 164 (July 25) 1936.

35. Emmerie. A.: On the Relation Between Intake and Excretion of Flavins, Acta brev. Neerland. **7,** 1937, Nos. 4, 5.

60 per cent of the normal amount. Roscoe[36] observed a similar train of events. With an increased intake there was both an increased retention and excretion. The rate of destruction of riboflavin by the body has not been reported, but the observations cited indicate that some destruction does occur. One might estimate from Emmerie's data that a man should receive from 2 to 3 mg. of riboflavin daily.

OVERDOSAGE OF RIBOFLAVIN NOT TOXIC

Kuhn and Boulanger[37] reported that mice were not injured by doses of 340 mg. of riboflavin per kilogram of body weight, roughly 1,000 times the daily requirement. If calculated on the same weight ratio, a man weighing 70 Kg. would not be injured by ingesting 20 Gm. of lactoflavin, the amount present in 20,000 liters of cow's milk.

RIBOFLAVIN INTAKE AND AMOUNT SECRETED IN MILK

The amount of vitamin G, either in cow's milk[38] or in human milk,[39] depends somewhat on the amount consumed, but precise data on this point are not available. It has been estimated[40] that a cow may consume 50 mg. of riboflavin in one day and secrete 10 liters of milk containing 1 mg. of riboflavin per liter, thus giving a recovery of 20 per cent. Each of these quantities is subject to revision, but they are probably of the correct order of magnitude.

Yellow fluorescence does not appear in the milk of wetnurses on a vegetarian diet[41] but is quite evident if the intake of riboflavin is increased. Müller[42] observed that after a diet of 250 Gm. of ox liver, which contained approximately 4 mg. of riboflavin, the yellow fluorescence approached that of cow's milk, provided the total milk production was low. If large amounts of milk were secreted, the flavin was correspondingly diluted. Müller also gave intramuscular injections of riboflavin and stated that 0.75 mg. of riboflavin was the smallest amount that would give a discernible yellow fluorescence. The amount of vegetables, even of spinach, that would contain this amount is far too great to be consumed in a reasonable time. Müller reported that children who received milk with some yellow fluorescence did not grow more rapidly than those who consumed milk with blue fluorescence, but the source of the fluorescence was not determined in either case. Because a riboflavin avitaminosis in man has not been reported, it is not known definitely that an increase in the riboflavin content of milk would be of clinical significance.

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[36] Roscoe, Margaret H.: The B Vitamins in Human Urine, Biochem. J. **30:** 1053 (June) 1936.

[37] Kuhn, Richard, and Boulanger, Paul: Ueber die Giftigkeit der Flavine, Ztschr. f. physiol. Chem. **241:** 233 (July 17) 1936.

[38] Hunt, C. H., and Krauss, W. E.: The Influence of the Ration of the Cow upon the Vitamin B and Vitamin G Content of Milk, J. Biol. Chem. **92:** 631 (Aug.) 1931.

[39] Donelson, Eva G., and Macy, Icie G.: Human Milk Studies: XII. The Vitamin B and Vitamin G Content Before and During Maternal Consumption of Yeast, J. Nutrition **7:** 231 (Feb.) 1934.

[40] Kuhn, Richard; Wagner-Jauregg, Theodor, and Kaltschmitt, Hans: Ueber die Verbreitung der Flavine im Pflanzenreich, Ber. d. deutschen chem. Gesellsch. **67:** 1452, 1934.

[41] Kayser, M. E.: Welcher Leberbestandteil geht in die Frauenmilch über? Deutsche med. Wchnschr. **63:** 136 (Jan. 22) 1937.

[42] Müller, R.: Beobachten über den Lactoflavingehalt der Frauenmilch und seine Beeinflussung durch die Ernährung, Klin. Wchnschr. **16:** 807 (June 5) 1937.

RIBOFLAVIN CONTENT OF THE EYE

Von Euler and Adler[43] reported that the retina of fish eyes contains flavin and suggested that this flavin is of special significance when the light is not intense. The pigment may make it possible to use shorter waves by changing them into green light, for which the eye has a maximum sensitivity. They had an opportunity to examine a human eye and obtained from it between 0.2 and 0.4 microgram of riboflavin. A codfish eye contains about 50 micrograms, or more than 100 times as much.

RIBOFLAVIN DEFICIENCY AND CATARACT

Cataract in rats is a consequence of vitamin G deficiency,[44] though some observers report that the condition occurs only in rats that are deprived of the vitamin at an early age.[45] Day and Langston[46] observed that a conjunctivitis and keratitis usually developed at

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[43] Karrer, P.; von Euler, Hans, and Schöpp, K.: Flavin aus Fischaugen, Ark. Kemi. Min. Geol. **11B,** 1935, No. 54. von Euler, Hans, and Adler, Erich: Ueber Flavin und einen blau-fluorescierenden Stoff in der Netzhaut der Fischaugen, Ztschr. f. physiol. Chem. **228:** 1 (Oct. 3) 1934. von Euler and Adler.[29]

[44] Day, P. L.; Langston, W. C., and O'Brien, C. S.: Cataract and Other Ocular Changes in Vitamin G Deficiency, Am. J. Ophth. **14:** 1005 (Oct.) 1931.

[45] O'Brien, C. S.: Experimental Cataract in Vitamin G Deficiency, Arch. Ophth. **8:** 880 (Dec.) 1932. Yudkin, A. M.: Ocular Disturbances Produced in Experimental Animals by Dietary Changes, J. A. M. A. **101:** 921 (Sept. 16) 1933.

[46] Day, P. L., and Langston, W. C.: Further Experiments with Cataract in Albino Rats Resulting from the Withdrawal of Vitamin G (B_2) from the Diet, J. Nutrition **7:** 97 (Jan.) 1934.

7 or 8 weeks, followed by a dulness of the eyeball and finally a definite opacity. The incidence of the cataract was only a little less than 100 per cent. Cataract was also produced in mice, chickens and monkeys.[47] Definite evidence that riboflavin is the vitamin G that prevents this type of cataract has been provided by Day, Darby and Langston.[48]

Bourne and Pyke[49] repeated Day's work, with a maximum incidence of cataract of 31 per cent. György[50] states that cataract was not observed, even though a large number of animals was observed, and in the experience of Richardson and Hogan[2] this abnormality was rare. The reason for the discrepancies is not apparent, but there is no reason to doubt that under suitable experimental conditions cataract in rats is caused by a deficiency of flavin.

Curiously enough, Mitchell and Dodge[51] have reported that cataract in rats is a consequence of a high lactose diet, but according to Morgan and Cook[52] the lactose type of dermatitis is not healed by riboflavin.

RIBOFLAVIN, CANINE BLACKTONGUE AND HUMAN PELLAGRA

The hypothesis that blacktongue in dogs is due to a deficiency of vitamin G has not been sustained,[53]

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47. Day, P. L.; Langston, W. C., and Cosgrove, K. W.: The Appearance of Cataract and Dermatitis in Experimental Animals Given Vitamin G Deficient Diets Containing Casein and Egg Albumin, J. Nutrition Proc. **7:** 12 (May) 1934. Day, P. L.: Vitamin G Deficiency, Am. J. Pub. Health **24:** 603 (June) 1934.

48. Day, P. L.; Darby, W. J., and Langston, W. C.: The Identity of Flavin with the Cataract-Preventive Factor, J. Nutrition **13:** 389 (April) 1937.

49. Bourne, Margherita C., and Pyke, M. A.: The Occurrence of Cataract in Rats Fed on Diets Deficient in Vitamin B_2, Biochem. J. **29:** 1865 (Aug.) 1935.

50. György, Paul: Investigations on the Vitamin B_2 Complex: I. The Differentiation of Lactoflavin and the "Rat Antipellagra" Factor, Biochem. J. **29:** 741 (March) 1935.

51. Mitchell, Helen S., and Dodge, W. M.: Cataract in Rats Fed on High Lactose Rations, J. Nutrition **9:** 37 (Jan.) 1935.

52. Morgan, Agnes F., and Cook, Bessie B.: Cataract- and Dermatitis-Producing Nutritional Factors, Proc. Soc. Exper. Biol. & Med. **34:** 281 (March) 1936.

53. Cowgill, G. R.; Zimmerman, H. M., and Burack, Ethel: Studies of Vitamin G (B_2) Deficiency in Dogs: Preliminary Communication, Am. J. Physiol. **109:** 24 (July) 1934. Zimmerman, H. M., and Burack, Ethel: Studies on the Nervous System in Deficiency Diseases: II. Lesions Produced in the Dog by Diets Lacking the Water-Soluble, Heat-Stable Vitamin B_2 (G), J. Exper. Med. **59:** 21 (Jan.) 1934. Zimmerman, H. M.; Cowgill, G. R.; Bunnell, W. W., and Dann, M.: Studies on the Nervous System in Deficiency Diseases: Experimental Black Tongue, Am. J. Physiol. **109:** 440 (Sept.) 1934. Rhoads, C. P., and Miller, D. K.: Vitamin B_2 (G) and Canine Blacktongue, Science **81:** 159 (Feb. 8) 1935.

and more recent studies[54] show that this disease is not prevented by riboflavin.

The earlier work of Cowgill and his collaborators[55] on neurologic manifestations in vitamin G (B_2) deficiency was repeated with some modifications, with about the same results. The neurologic changes are described as (*a*) marked degeneration of the medullary sheaths and axis cylinders of the peripheral nerves, (*b*) fairly common degeneration of the medullary sheaths of the posterior nerve roots, with less frequent involvement of the anterior roots of the cord, and (*c*) degeneration of the medullary sheaths and axis cylinders of the posterior columns of the spinal cord.

The authors did not show that riboflavin would prevent the lesions they ascribed to a deficiency of G (B_2) but in a personal communication dated June 30, 1937, Dr. Cowgill stated that they were investigating that specific point, and that up to that date riboflavin afforded the same degree of protection against the vitamin G deficiency syndrome as did the liver extract previously used. Rats on diets similar to those employed by Zimmerman and Burack do not develop degeneration of the myelin sheaths in the spinal cord or in peripheral nerves, so dogs and rats seem to respond differently to this deficiency.[56] Wolbach's attitude is apparently expressed in the statement that "the demonstrable nervous lesions of all the deficiency diseases are secondary effects" and "no one vitamin is concerned in the maintenance of myelin."

Sebrell, Hunt and Onstott[57] confirmed the fact that riboflavin has no therapeutic value in the treatment of blacktongue. It was demonstrated by Richardson and Hogan[2] that Goldberger's[58] blacktongue producing diet contains suboptimal amounts of flavin, and Sebrell,

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[54] Birch, T. W.; György, Paul, and Harris, L. J.: The Vitamin B_2 Complex: Differentiation of the Antiblacktongue and the "P.-P." Factors from Lactoflavin and Vitamin B_6 (So-Called "Rat Pellagra" Factor): Parts I-IV, Biochem. J. **29:** 2830 (Dec.) 1935. Koehn, C. J., and Elvehjem, C. A.: Studies on Vitamin G (B_2) and Its Relation to Canine Black Tongue, J. Nutrition **11:** 67 (Jan.) 1936.

[55] Zimmerman, H. M.; Cowgill, G. R., and Fox, J. C., Jr.: Neurologic Manifestations in Vitamin G (B_2) Deficiency: An Experimental Study in Dogs, Arch. Neurol. & Psychiat. **37:** 286 (Feb.) 1937.

[56] Wolbach, S. B.: The Pathologic Changes Resulting from Vitamin Deficiency, J. A. M. A. **108:** 7 (Jan. 2) 1937.

[57] Sebrell. W. H.; Hunt, D. J., and Onstott, R. H.: Lactoflavin in the Treatment of Black Tongue, Pub. Health Rep. **52:** 235 (Feb. 26) 1937.

[58] Goldberger, Joseph, and Wheeler, G. A.: Experimental Black Tongue of Dogs and Its Relation to Pellagra, Pub. Health Rep. **43:** 172 (Jan. 27) 1928.

Onstott and Hunt[59] observed that this deficiency may be significant. Thus one dog that had been healed of blacktongue with the filtrate factor collapsed 182 days from the beginning of treatment. During one day 8 mg. of lactoflavin was injected intramuscularly and within twelve hours after the first injection the animal was normal. Evidently the dog requires flavin, and a ration could be compounded of natural foodstuffs which is deficient in this substance.

Dann[60] has reported that riboflavin is ineffective in the treatment of human pellagra. Fouts, Lepkovsky, Helmer and Jukes[61] treated two pellagrins with the filtrate factor of Lepkovsky, Jukes and Krause,[62] and reported that they were healed. This filtrate factor was prepared from an extract from which lactoflavin had been removed.

Evidently a deficiency of riboflavin is not the primary cause of either pellagra or blacktongue, but it is entirely possible that such a deficiency may be a complication. The diets that are commonly consumed in pellagrous districts are poor sources of riboflavin.[62a]

CHICK PELLAGRA

Chicks also develop "pellagra" symptoms[63] when reared on rations that have been subjected to prolonged dry heat, but the symptoms are not due to a deficiency of flavin.[64]

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[59] Sebrell, W. H.; Onstott, R. H., and Hunt, D. J.: The Treatment of Blacktongue with a Preparation Containing the "Filtrate Factor," and Evidence of Riboflavin Deficiency in Dogs, Pub. Health Rep. **52:** 427 (April 9) 1937.

[60] Dann, W. J.: The Vitamin G Complex: I. The Nonidentity of Rat Dermatitis Due to Vitamin B_6 Deficiency and the Dermatitis of Human Pellagra, J. Nutrition **11:** 451 (May) 1936.

[61] Fouts, P. J.; Lepkovsky, Samuel; Helmer, O. M., and Jukes, T. H.: Successful Treatment of Human Pellagra with the "Filtrate Factor," Proc. Soc. Exper. Biol. & Med. **35:** 245 (Nov.) 1936.

[62] Lepkovsky, Samuel; Jukes, T. H., and Krause, Myrtise E.: The Multiple Nature of the Third Factor of the Vitamin B Complex, J. Biol. Chem. **115:** 557 (Sept.) 1936.

[62a] After this manuscript had been completed, it was demonstrated that nicotinic acid prevents, and heals, both blacktongue and pellagra. Elvehjem, C. A.; Madden, R. J.; Strong, F. M., and Wooley, D. W.: Relation of Nicotinic Acid and Nicotinic Acid Amid to Canine Black Tongue, J. Am. Chem. Soc. **59:** 1767 (Sept.) 1937. Fouts, P. J.; Helmer, O. M.; Lepkovsky, S., and Jukes, T. H.: Treatment of Human Pellagra with Nicotinic Acid, Proc. Soc. Exper. Biol. & Med. **37:** 405 (Nov.) 1937. Smith, D. T.; Ruffin, J. M., and Smith, S. G.: Pellagra Successfully Treated with Nicotinic Acid, J. Am. Med. Assoc. **109:** 2054 (Dec.) 1937.

[63] Kline, O. L.; Keenan, J. A.; Elvehjem, C. A., and Hart, E. B.: The Use of the Chick in Vitamin B_1 and B_2 Studies, J. Biol. Chem. **99:** 295 (Dec.) 1932. Lepkovsky, Samuel, and Jukes, T. H.: The Vitamin G Requirement of the Chick, ibid. **111:** 119 (Sept.) 1935. Ansbacher, S.; Supplee, G. C., and Bender, R. C.: Pellagra-like Syndrome in Chicks, J. Nutrition **11:** 529 (June) 1936.

[64] Elvehjem, C. A., and Koehn, C. J., Studies on Vitamin B_2 (G): The Nonidentity of Vitamin B_2 and Flavins, J. Biol. Chem. **108:** 709 (March) 1935.

RIBOFLAVIN AND PERNICIOUS ANEMIA

Preliminary observations of Strauss and Castle[65] on the factor that prevents pernicious anemia led them to suggest that the extrinsic factor of pernicious anemia is vitamin B_2 (G) or is closely related to it, but practically all of the more recent reports are negative. Wills and Naish[66] used an extract of egg white as a source of vitamin G and incubated it with normal gastric juice and supplied it to a patient with pernicious anemia, but the outcome was negative. It was shown that same year that egg white contains riboflavin; therefore this newly discovered compound could not be the extrinsic factor. This view has been confirmed by numerous other workers.[67]

Stare and Thompson[68] administered riboflavin intramuscularly to five patients with pernicious anemia, with no effect. Ashford, Klein and Wilkinson[69] incubated riboflavin with normal gastric juice, and also with the press juice of hog's stomach, but the hematopoietic activity of the flavin was not increased by the treatment. According to these investigators riboflavin is not the extrinsic factor and is not the antipernicious anemia principle.

RIBOFLAVIN, ADRENAL CORTEX EXTRACT AND IODOACETIC ACID POISONING

Laszt and Verzár[70] supplied to rats a complete diet, to which had been added 0.02 per cent iodoacetic acid. This was consumed readily, but growth was retarded and after a time the rats became apathetic, there were alterations in the bones, skin and blood, and the adrenals were hypertrophic. If the rats were given 20 micrograms daily of riboflavin-phosphoric acid, growth was restored, but riboflavin itself was ineffective. If, instead of the riboflavin ester, the rats received injections of the adrenal cortex extract, growth was again resumed. From these observations the authors concluded that riboflavin must be phosphorylated before it can exercise its normal physiologic function, and that adrenal cortex extract is essential for the phosphorylation process. The toxicity of iodoacetic acid is largely due to the fact that it inhibits this ester formation. They published additional evidence [71] on this point and suggest that there is a similarity between iodoacetic acid poisoning and the Gee-Herter syndrome (celiac disease).[72] They believe that in both cases the formation of riboflavin phosphoric acid is inhibited. Rudy [73] also has studied iodoacetic acid poisoning of rats, but he observed that simple riboflavin is as effective in restoring growth as the phosphoric acid ester. The explanation of this discrepancy is not apparent.

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[65] Strauss, M. B., and Castle, W. B.: The Nature of the Extrinsic Factor of the Deficiency State in Pernicious Anemia and in Related Macrocytic Anemias, New England J. Med. **207:** 55 (July 14) 1932.

[66] Wills, Lucy, and Naish, Alice: A Case of Pernicious Anemia Treated with Vitamin B_2 from Egg White, Lancet **1:** 1286 (June 17) 1933.

[67] Lassen, H. C. A., and Lassen, H. K.: Yeast or Vitamin B_2 as "Extrinsic Factor" in Treatment of Pernicious Anemia, Am. J. M. Sc. **188:** 461 (Oct.) 1934. Brand, Erwin; West, Randolph, and Stuckey, C. J.: Vitamin G Potency of Purified Liver Preparations, Proc. Soc. Exper. Biol. & Med. **30:** 1382 (June) 1933. Miller, D. K., and Rhoads, C. P.: The Presence in Egg-White and in a Rice Polishings Concentrate Low in Vitamin B_2 (G) of an Anti-Pernicious Anemia Principle, New England J. Med. **211:** 921 (Nov. 15) 1934.

[68] Stare, F. J., and Thompson, L. D.: Hepatoflavin and Pernicious Anemia. Proc. Soc. Exper. Biol. & Med. **33:** 64 (Oct.) 1935.

[69] Ashford, C. A.; Klein, Louis, and Wilkinson, J. F.: Note on the Nonidentity of Lactoflavin and the "Extrinsic Factor" in Pernicious Anaemia, Biochem. J. **30:** 218 (Feb.) 1936.

[70] Laszt, L., and Verzár, F.: Hemmung des Wachstums durch Jodessigsäure und antagonistische Beeinflussung durch Vitamin B_2 sowie Nebennierenrinden-Hormon, Arch. f. d. ges. Physiol. (Pflüger's) **236:** 693 (Dec. 23) 1935

Adrenalectomy interferes with fat absorption, but Laszt and Verzár [74] report that absorption is restored to the normal level by administering either adrenal cortex extract or riboflavin phosphoric acid.

RIBOFLAVIN IN AVIAN NUTRITION

Lepkovsky and Jukes [75] reported that chicks which received a basal diet which was deficient in flavin grew slowly, became weak and emaciated and developed diarrhea but did not develop dermatitis. Controls that received riboflavin were normal. Turkeys did not react in exactly the same way. After the eighth day a dermatitis developed which resembled the deficiency disease of chicks first described by Ringrose, Norris and Heuser.[76] If the basal diet was supplemented with riboflavin in the proportion of 2 mg. of the vitamin per hundred grams of the diet, dermatitis did not develop, there was no mortality and growth was rapid. There was no mention of leg weakness in either chicks or turkeys.

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[71] Verzár, F., and Laszt, L.: Der Zusammenhang zwischen Vitamin B_2 und dem Hormon der Nebennierenrinde, Arch. f. d. ges. Physiol. (Pflüger's) **237:** 476 (May 18) 1936.

[72] Laszt, L., and Verzár, F.: Ueber chronische Jodessigsäurevergiftung und ihre Beziehung zur Gee-Herterschen Krankheit, Arch. f. d. ges. Physiol. (Pflüger's) **237:** 483 (May 18) 1936.

[73] Rudy, Hermann: Ueber die Wachstumswirkung von Lactoflavinphosphorsäure und gelbem Ferment, Ztschr. f. angew. Chem. **49:** 323, 1936.

[74] Laszt, L., and Verzár, F.: Nebennierenrinde und Fettresorption, Biochem. Ztschr. **228:** 351 (Dec. 12) 1936.

[75] Lepkovsky, Samuel, and Jukes, T. H.: The Response of Rats, Chicks, and Turkey Poults to Crystalline Vitamin G (Flavin), J. Nutrition **12:** 515 (Nov.) 1936.

[76] Ringrose A. T.; Norris, L. C., and Heuser, G. F.: The Occurrence of a Pellagra-like Syndrome in Chicks, Poultry Sc. **10:** 166, 1931.

Bethke, Record and Wilder [77] provided baby chicks with a basal ration that was deficient in flavin and observed that it caused a characteristic leg disorder as well as a subnormal rate of growth. When the basal diet was supplemented with 40 micrograms of riboflavin every other day the weights were normal and there were no leg disorders. The ration used by Bethke and his collaborators was not strikingly different from diet 60 supplied by Lepkovsky and Jukes to turkeys, so it is not clear why the former reported a high incidence of leg weakness and the latter reported none at all.

The amount of vitamin G in the ration of laying hens determines, within limits, the amount in the eggs,[78] and this amount in turn determines the hatchability of the eggs.[79]

HUMAN RIBOFLAVIN DEFICIENCY

In a preliminary note Sebrell and Butler [79a] recently described induced riboflavin deficiency in man. Ten of eighteen women on a diet deficient in riboflavin developed macerated areas in each angle of the mouth which progressed to transverse fissuring. The mucosa of the lips became shiny, abnormally red, and had a denuded appearance. There were also greasy, seborrheic accumulations at the alae nasae, around the eyes, and in some instances on the ears. The lesions disappeared from four patients after the oral administration of synthetic crystalline riboflavin. In the remaining six patients the lesions failed to respond to treatment with nicotinic acid but disappeared after treatment with riboflavin. Vilter, Vilter and Spies[79b] have seen beneficial effects from riboflavin in four pellagrins whose skin lesions had disappeared under nicotinic acid therapy but who still had vague and indefinite symptoms of ill health. They also found that it was not necessary to increase greatly the dose of nicotinic acid to prevent recurrence of symptoms if riboflavin was given. Naturally occurring riboflavin deficiency with lesions identical to those described by Sebrell and Butler has been observed in adults and children by several investigators. The content of coenzyme I and II in the blood of patients with leukemia has been found low by Vilter, Vilter and Spies.[79c] They have observed temporary return of the coenzyme to normal levels following the daily ingestion of 100 Gm. of brewers' yeast and 5 mg. of synthetic riboflavin.

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[77] Bethke, R. M.; Record, P. R., and Wilder, O. H. M.: Further Studies on Vitamin G in Chick Nutrition with Special Reference to Flavins, Poultry Sc. **16**: 175, 1937.

[78] Bethke, R. M.; Record, P. R., and Wilder, F. W.: The Effect ot the Ration of the Hen on the Vitamin G Content of Eggs, with Observations on the Distribution of Vitamin B and G in Normal Eggs, J. Nutrition **12**: 309 (Sept.) 1936. Norris, L. C.; Wilgus, H. S.; Ringrose. A. T.; Heiman, V., and Heuser, G. F.: The Vitamin G Requirement of Poultry, Bull. 660, Cornell Agric. Exper. Sta., 1936.

[79] Ellis, N. R.; Miller, David; Titus, H. W., and Byerly, T. C.: Effect of Diet on Egg Composition: III. The Relation of Diet to the Vitamin B and the Vitamin G Content of Eggs, Together with Observations on the Vitamin A Content, J. Nutrition **6**: 243 (May) 1933. Bethke, R. M.; Record, P. R., and Kennard, D. C.: The Relation of Vitamin G to the Hatchability of Hens' Eggs, J. Nutrition **12**: 297 (Sept.) 1936. Norris, Wilgus, Ringrose, Heiman and Heuser.[78]

[79a] Sebrell, W. H., and Butler, R. E.: Riboflavin Deficiency in Man. A Preliminary Note, Pub. Health Repts. **53**: 2282-2284 (Dec. 30) 1938.

MISCELLANEOUS

When rats receive diets that are deficient in vitamin G the oxygen uptake of skin cells is lowered.[80] The percentage of dry matter and of total fat in the tissues is markedly reduced, and that of water correspondingly increased, but these symptoms may be due merely to inanition.[81]

If the heart is perfused under low oxygen pressure the muscular contractions are weakened.[82] A normal heart beat is restored either by physiologic oxygen pressure or by adding a minute quantity of riboflavin to the perfusion medium.

It has been reported[83] that strenuous muscular exercise may cause a hypertrophy of the adrenal glands of rats, amounting to 82 per cent. The hypertrophy was prevented by feeding large amounts of autoclaved

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79b. Vilter, Richard W.; Vilter, Sue Potter, and Spies, Tom D.: Relationship Between Nicotinic Acid and a Codehydrogenase (Cozymase), J. A. M. A. **112**: 420-422 (Feb. 4) 1939.

79c. Vilter, Richard, W.; Vilter, Sue Potter, and Spies, Tom D.: Determination of Codehydrogenases I and II (Cozymase) in Blood of Diabetics in Severe Acidosis, Am. J. Med. Sc. **197**: 322-326 (March) 1939.

80. Adams, P. D.: The Oxygen Uptake and Composition of the Skin of Rats in Vitamin G Deficiency, J. Biol. Chem. **116**: 641 (Dec.) 1936.

81. Remp, D. G., and Bing, F. C.: Inanition as a Factor in Vitamin G Deficiency, J. Nutrition **8**: 457 (Oct.) 1934.

82. Dietrich, S., and Pendl, E.: Vitamin B_2 (Lactoflavin) and Erstickung des isolierten Froschherzens, Klin. Wchnschr. **16**: 13 (Jan. 2) 1937.

83. von Beznák, A., and Perjés, J.: Ueber den Zusammenhang der Nebennierenrindenhypertrophie mit der körperlichen Arbeit und mit dem Gehalt der Nährung an Vitamin B, Arch. f. d. ges. Physiol. (Pflüger's) **236**: 181 (Sept. 14) 1935.

yeast but not by administering riboflavin.[84] It was suggested that the activity of yeast was due to its content of vitamin B_6.

A deficiency of vitamin G has little or no effect on the concentration of tissue enzymes.[85]

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[84] *Perjés, J.: Weitere Untersuchungen über die wirkung einzelner Glieder des Vitamin B-Komplexes auf die durch körperliche Arbeit bedingte Volumzunahme der Nebennierenrinde,* Arch. f. d. ges. Physiol. (Pflüger's) **238:** 341 (Dec. 18) 1936.

[85] Kik, M. C.: Sure, Barnett, and Buchanan, Kathryn Sue: Enzymic Efficiency in Avitaminosis: III. Influence of Vitamin B_1 and G Deficiencies on the Concentration of Blood and Tissue Enzymes, Am. J. Digest. Dis. & Nutrition **3:** 491 (Sept.) 1936.

CHAPTER XV

RIBOFLAVIN

DIETARY SOURCES AND REQUIREMENTS

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AND
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At the time of the preceding symposium in 1932, the terms vitamin B_2 and G were used synonymously by Sure[1] as standing for the more heat stable part of the vitamin B complex; and Underhill[2] wrote of vitamin G deficiency as "intimately associated with the complex syndrome of symptoms included under the term pellagra." At the time of the present writing (the summer of 1937) the evidence appears conclusive that there are at least three relatively heat stable substances in the vitamin B complex: (1) riboflavin; (2) vitamin B_6; and (3) another substance which perhaps may be considered P-P or the Goldberger pellagra-preventive substance, which may or may not be the same as the substance which prevents blacktongue in dogs. (It is also held by some careful students that more than one substance may be involved in the nutritional problem of human pellagra, even when this is distinguished from all so-called pellagra-like disorders in other species.)

Riboflavin and vitamin B_6 are both required for growth and for the maintenance of health in the rat and probably in mammals generally, including man. The fact that we do not know any specific human disease due to shortage of riboflavin is entirely compatible with the view that this substance is important in human nutrition. A detailed discussion of reasons for believing that riboflavin plays a rôle in the life process of the human as of other species would probably seem superfluous to a majority of readers at this date, and to a

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From the Department of Chemistry, Columbia University.

[1] Sure, Barnett: The Present Status of Vitamin B_2 (G), J. A. M. A. **99:** 26 (July 2) 1932.

[2] Underhill, F. P.: Clinical Aspects of Vitamin G Deficiency, J. A. M. A. **99:** 120 (July 9) 1932.

still larger majority in the future. Suffice it to point out that our species has evolved in the direction not of shortening the list of things it needs but of lengthening the list of things it can use to advantage.

Shortage of riboflavin stunts the growth of the young and at any age causes a lowering of general tone and a condition of premature aging or unwholesomeness of the skin, with loss of hair. The skin condition has sometimes been called a "nonspecific" dermatitis or dermatosis as distinguished from the "florid" dermatitis of vitamin B_6 deficiency.[3]

The substance here called riboflavin was first described as lactoflavin (previously called lactochrome).[4] Ovoflavin and hepatoflavin, first so named for the materials from which they were prepared, were soon found to be chemically identical with lactoflavin.

The same substance has also been reported as isolated from several other sources as widely separated biologically as the milk of different species, muscle, liver, kidney, eggs, fish eyes, malt, grass, dandelion flowers, and both marine and fresh water algae as typified by Fucus vesiculosus and Cladophora sauteri (Karrer;[5] Heilbron, Parry and Phipers[6]).

DIETARY SOURCES OF RIBOFLAVIN

Corresponding with the wide biologic distribution of riboflavin in the plant and animal kingdoms as just mentioned, it is found more generally present in significant amounts among varied food materials than is vitamin A but less abundantly in the seeds which form so large a part of man's food than is vitamin B_1. It is doubtless true, as suggested by Eusterman and Wilbur[7] in the previous symposium, "that certain individuals, the poor in particular, do not consume enough foods that contain [significant amounts of] vitamin A and vitamin G to insure a state of nutrition essential to the greatest physical stamina, efficiency and ability to resist infectious diseases." In fact, this view is strongly reenforced by the experimental finding[8] that the optimal intake of vitamin G (riboflavin) is very much (probably at least fourfold) higher than is easily demonstrable as strictly necessary.

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[3] Chick, Harriet; Copping, Alice M., and Edgar, Constance E.: The Components of Vitamin B_2, Biochem. J. **29:** 722 (March) 1935.

[4] Booher, Lela E.: Concentration and Chemical Nature of Vitamin G, J. Biol. Chem. **107:** 591 (Nov.) 1934.

[5] Karrer, P.: Nutritionally Important Natural Pigments, Helv. Chim. Acta **19:** E 33 (special number) 1936.

[6] Heilbron, I. M.; Parry, E. G., and Phipers, R. F.: The Algae: III. The Isolation of Lactoflavin, Biochem. J. **29:** 1382 (June) 1935.

[7] Eusterman, G. B., and Wilbur, D. L., in The Vitamins: A Symposium on the Present Status of the Knowledge of Vitamins, Chicago, American Medical Association, 1932, p. 25.

The relative amounts of riboflavin in foods are therefore a matter of importance to health and should be given weight in present-day considerations of food values and planning of the dietary.

TABLE 1.—*Approximate Riboflavin Values of Certain Foods* *

	Units† per 100 Gm
Apples	10 to 43
Bananas	26 to 50
Beef, lean	90 to 150
Carrots	30 to 75
Carrot tops	200
Cheese	44 to 288
Cottonseed flour or meal	170 to 300
Eggs	99 to 150
Egg white	60 to 120
Egg yolk	150 to 300
Kale	140 to 260
Liver	800 to 1,200
Milk	34 to 100
Oranges	15 to 65
Potatoes	15 to 31
Spinach	100 to 175
Tomatoes	12 to 28
Turnips	17 to 50
Turnip greens	300
Wheat, entire	48 to 124
Wheat germ	150 to 404
Yeast, dry	750 to 2,500

* Selected from among the data on pages 415 to 417 of Sherman's Chemistry of Food and Nutrition, ed. 5, 1937, from Daniel and Munsell[10] and from Levine and Remington.[11]

† These units are Sherman-Bourquin units of vitamin G determined by the rat-growth method of Bourquin and Sherman[13] and of Page,[19] as more fully explained below.

From longer lists published elsewhere[9] and from data reported by Daniel and Munsell[10] we selected for table 1 some typical foods, including those which have thus far been most thoroughly studied in this respect.[11] Some of these deserve additional brief comment.

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[8] Sherman, H. C., and Ellis, Lillian N.: Necessary versus Optimal Intake of Vitamin G, J. Biol. Chem. **104**: 91 (Jan.) 1934.

[9] Sherman, H. C.: Chemistry of Food and Nutrition, ed. 5, New York, Macmillan Company, 1937.

[10] Daniel, Esther P., and Munsell, Hazel E.: Vitamin Content of Foods, U. S. Dept. of Agriculture, Misc. Pub. No. 275.

[11] Levine, Harold, and Remington, R. E.: The Vitamin G Content of Some Foods, J. Nutrition **13**: 525 (May) 1937.

Riboflavin appears to be formed primarily in the green leaves of actively growing plants and to remain there in higher concentration than elsewhere in the plant. Thus, of the three tissues constituting the edible portion of broccoli, the flower buds contain only a little over half as much riboflavin as the leaves, and the twigs still less. Similarly, carrot tops show something like four times the riboflavin value of the roots. This concentration of riboflavin in the green leaves where it is formed presents an interesting contrast to the transfer of carotene from leaf to root which occurs in the carrot.

There are indications that, as the leaves mature and dry, there may be a considerable diminution in their riboflavin value. Correspondingly it was found at the Ohio Agricultural Experiment Station [12] that milk from cows receiving an abundance of fresh young grass tended to be slightly richer in riboflavin than the milk of cows fed drier and more mature grass; but in milk as ordinarily produced under present-day conditions of dairy farming seasonal variations of vitamin value are much smaller than would be inferred from many of the statements in the literature. Perhaps the best present evidence in this case is expressed in the figures of the seventeen available quantitative results of ten independent investigations. These show an average of 67 ± 3 Bourquin-Sherman [13] units of riboflavin per hundred grams of milk, with a coefficient of variation of 28, which is a lesser variability than would perhaps have been expected. Nor should there be any serious diminution of the riboflavin value of milk in pasteurization or in preservation by drying or by condensation (partial evaporation) and canning. Milk is thus a relatively stable and dependable source of riboflavin and will almost always be the chief source in the dietaries of children because of the prominence that should be given to milk in the dietary for other nutritional reasons as well. In any long view of food economics there is also the consideration that milk is a food crop the production of which can be greatly increased in response to consumer demand, with little if any increase in cost per unit to the consumer.

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[12] Hunt, C. H., and Krauss, W. E.: The Influence of the Ration of the Cow upon the Vitamin B and Vitamin G Content of Milk, J. Biol. Chem. **92**: 631 (Aug.) 1931.

[13] Bourquin, Anne, and Sherman, H. C.: Quantitative Determination of Vitamin G, J. Am. Chem. Soc. **53**: 3501 (Sept.) 1931.

A contrasting case in this respect is that of liver, the supply of which can be increased only by rearing and slaughtering whole animals of which the liver is only a very minor part. Hence (as Minot, if we remember correctly, has complained) any emphasis on liver as a food for people in general has the unfortunate result of increase in its cost to those who really need it.

György [14] has made semiquantitative comparisons of the riboflavin values of some different flesh foods. He finds fish muscle a poor source of riboflavin, compared with beef and chicken muscle, which he finds to contain about equal concentrations. The red leg muscle of chicken appeared to contain slightly more than the white breast muscle; but this difference does not appear sufficient to justify discrimination in a practical consideration of dietary sources. In György's experiments, beef liver appeared about fifteenfold richer than beef muscle, while in the apparently more precise experiments of Day [15] and of Munsell [10] liver showed about tenfold higher riboflavin value than did an equal weight of muscle.

Rose and Phipard [16] recently found that fresh raw peas and lima beans contain about 100 units of riboflavin per hundred grams and observed no loss in either cooking or freezing. In mature seeds of both kinds there appeared to be little diminution in vitamin G (riboflavin) value as compared with the fresh seeds. Pea seedlings showed an increase in riboflavin content over the seeds from which they were sprouted.

In practice, the actual importance of any given food as a dietary source of riboflavin (or of any other specific nutrient) depends of course not only on the concentration of the substance in the food but also on the place (or quantitative prominence) of the food in the dietary or food supply. Stiebeling [17] has ascertained the relative place of different dietary articles in food budgets and has computed what proportion each contributes of the total of each of several nutrients thus secured. The data for riboflavin and some of the other nutrients are given in abbreviated form in table 2.

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[14] György, Paul: The Distribution of Lactoflavin (and of Vitamin B_6) in Natural Products of Animal Origin, Biochem. J. **29**: 760 (March) 1935.

[15] Day, P. L.: Vitamin G in Beef and Veal, J. Home Economics **23**: 657, 1931.

[16] Rose, Mary S., and Phipard, Esther H. F.: Vitamin B and G Values of Peas and Lima Beans Under Various Conditions, J. Nutrition **14**: 55 (July) 1937.

[17] Stiebeling, Hazel K.: Serial No. R 409, Bureau of Labor Statistics, U. S. Dept. Labor, 1936.

It seems sufficiently established that the Bourquin-Sherman method for the measurement of "vitamin G values" measures riboflavin rather than any other factor—and consistently so when the method is carried out strictly as described by its authors. This has been found independently by several users of the method but, of course, should not be assumed for any cases in which the method is modified, especially as to the preparation and use of the 80 per cent alcohol extract of wheat as a source of vitamin B_1, B_6 and any still unknown "B factors" needed by the rat. Even when the Bourquin-Sherman directions are explicitly followed, the adequacy of the extract to this purpose should be tested by each laboratory on the materials (and test animals) which it uses; for the vitamin B_6 value of wheat is reported to be subject to wide variation as tested in different parts of the world.[18] The amount

TABLE 2.—*Relative Prominence of Certain Types of Food* (in Dietaries Costing from $2.38 to $3 Weekly per Food-Cost Unit)*

Food or Food Group	Per Cent of Food Money Allocated	Percentages Contributed by Each Food Group to the Total Secured					
		Calories	Protein	Calcium	Vitamin A	Vitamin C	Riboflavin
Meats and fish	25.5	12.9	36.2	2.7	7.1	0.7	32.2
Eggs	5.0	1.7	4.9	2.7	6.0	...	5.3
Milk, cheese, ice cream	12.1	9.8	16.0	60.7	13.9	5.3	34.2
Butter, cream	7.6	9.3	0.4	1.4	16.0	?	0.4
Other fats	3.0	7.8	0.5	0.1	0.8	...	1.1
Breadstuffs, cereals, bakery products	17.6	30.3	27.3	12.0	3.5	0.6	5.7
Sugar, sweets	4.2	12.4	0.1	2.1	...	...	...
Potatoes, sweet potatoes	1.5	5.8	4.4	3.4	2.6	22.8	7.2
Dried legumes, nuts	1.8	2.9	5.3	3.4	0.2	...	0.8
Tomatoes	1.5	0.3	0.4	0.5	9.5	7.8	0.6
Citrus fruits	3.2	1.1	0.5	3.0	0.6	29.6	3.3
Green and yellow vegetables	3.9	0.8	1.4	3.4	33.7	12.8	3.7
Other vegetables	3.4	1.0	1.0	2.8	1.3	8.1	1.2
Other fruits	3.9	2.8	0.7	1.4	4.6	12.3	3.9

* Adapted from Stiebeling.[17]

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[18] György, Paul: Investigations on the Vitamin B_2 Complex: I-III, Biochem. J. **29**: 741 (March) 1935. Halliday, Nellie, and Evans, H. M.: Dietary Production of the Syndrome of Deficiency of Vitamin B_6, J. Nutrition **13**: 657 (June) 1937.

of riboflavin which the Bourquin-Sherman "unit of vitamin G" represents has been estimated as from about 3 to 5 micrograms. The chief causes of this variation, so far as present knowledge suggests, are (1) differences in purity of previously available preparations of riboflavin, (2) variations of riboflavin requirements with the size of the test animal, (3) completeness of prevention of coprophagy,[19] and (4) the possible influence of some less well defined difference in the nutritional background of the test animal or in the bacteriology of its environment, which may influence the precise amount of riboflavin needed or absorbed with a given diet, even when all mechanical precautions for prevention of coprophagy are observed.

DIETARY REQUIREMENT FOR RIBOFLAVIN

As yet, estimates of the amounts of riboflavin required for normal human nutrition are necessarily matters of judgment rather than of actual direct measurement. The independent judgments of Rose[20] and of Stiebeling[21] are in substantial agreement and are probably the most influential estimates thus far available. Rose has suggested for children up to 10 years of age at least 400 units a day in all cases, or 20 units per hundred calories if more than 2,000 calories a day is consumed; for adults, 20 units per hundred calories. The recommendation of Stiebeling is 450 units for boys under 6 and girls under 7 years of age, 540 units for boys from 7 to 10 and girls from 8 to 13 years of age, and 600 units for older children and adults, or 570 units per capita of the population.

How far above these allowances the actual optimum will be found is a question for the future.

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[19] Page, J. W.: An Improvement in Experimental Method for Investigation of Vitamin G, Proc. Soc. Exper. Biol. & Med. **30**: 87 (Oct.) 1932.

[20] Rose, Mary S.: Laboratory Handbook for Dietetics, ed. 4, New York, Macmillan Company, 1937.

[21] Stiebeling, Hazel K.: Publications of the U. S. Dept. Agriculture, 1936, and personal communication to the authors in April 1937.

CHAPTER XVI

VITAMINS IN RELATION TO THE PREVENTION AND TREATMENT OF PELLAGRA

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The exact number of vitamins or dietary factors in the so-called water soluble vitamin B complex that are essential to the human organism is still undetermined. There are four postulated factors about which sufficient evidence exists to warrant discussion in connection with the treatment and prevention of pellagra. These are riboflavin, or vitamin B_2(G), the rat antidermatitis factor or vitamin B_6, the filtrate factor or chick pellagra factor, and the pellagra-preventive vitamin or blacktongue-preventive factor. The field is being investigated so actively at the present time that it is almost impossible to make any conclusive statement concerning the identity of factors which have not been chemically isolated.

RIBOFLAVIN OR VITAMIN B_2(G)

There is considerable evidence that riboflavin is not the pellagra-preventive vitamin. Rhoads and Miller[1] reported that they were unable to produce blacktongue with a diet devoid of vitamin G and inferred that blacktongue is due to some factor other than vitamin G. Birch, György and Harris[2] failed to cure blacktongue with 30 micrograms of riboflavin per kilogram of body weight per day intraperitoneally, and György[3] noted that salmon and haddock, which are good sources of the pellagra-preventive vitamin, were practically devoid of riboflavin.

From the National Institute of Health, United States Public Health Service.

[1] Rhoads, C. P., and Miller, D. K.: Vitamin B_2 (G) and Canine Black Tongue, Science **81:** 159 (Feb. 8) 1935.

[2] Birch, T. W.; György, Paul, and Harris, L. J.: Vitamin B_2 Complex: Differentiation of Antiblacktongue and "P.-P." Factors from Lactoflavin and Vitamin B_6 (So-Called "Rat Pellagra" Factor), Biochem. J. **29:** 2830 (Dec.) 1935.

[3] György, Paul: Investigations on Vitamin B_2 Complex: Distribution of Lactoflavin and of "Pellagra-Preventing Factor" (Vitamin B_6) in Natural Products of Animal Origin, Biochem. J. **29:** 760 (No. 3) 1935.

Dann[4] treated three cases of pellagra with riboflavin without success. Fouts, Lepkovsky, Helmer and Jukes[5] treated two cases with riboflavin without success and then successfully treated two other cases with a preparation free from riboflavin. Koehn and Elvehjem[6] failed to prevent blacktongue with a flavin supplement, and Sebrell, Hunt and Onstott[7] were unable to cure blacktongue with relatively very large doses of a synthetic riboflavin preparation.

Although riboflavin may not be concerned in the etiology of pellagra, the foregoing evidence must not be interpreted as indicating that riboflavin may not be one of the human dietary essentials. The use of riboflavin in the treatment of neurologic lesions in a case of pernicious anemia has been reported by Kisch.[8] Sebrell[9] reported in 1933 the experimental production of a condition in dogs which, in view of evidence recently presented by Sebrell, Onstott and Hunt,[10] was due, very probably, to riboflavin deficiency. It is not impossible that riboflavin deficiency may occur in man simultaneously with, or independently of, pellagra.

VITAMIN B_6, OR THE RAT ANTIDERMATITIS FACTOR

The exact status of vitamin B_6 has not yet been finally determined. György[11] and Chick, Copping and Edgar[12] have suggested that it may be identical with the Y factor of Chick and Copping.[13] Jukes[14] still questions the accuracy of the work on vitamin B_6 and states: "The demonstration of two new factors necessary for the rat, in the vitamin B complex, in addition to vitamin B and flavin, has made it difficult to interpret recent work on the vitamin B complex, particularly with regard to vitamin B_6, which is probably a mixture of the two new factors." There is evidence that vitamin B_6 is distinct from the pellagra-preventive vitamin, and it is now recognized that what has been called rat pellagra in all probability bears no relationship to human pellagra, and that the skin lesions in these experimental animals are due to a deficiency either in riboflavin (vitamin B_2[G]), or vitamin B_6, or both. Birch, György and Harris[2] found that pellagra and blacktongue producing diets contained vitamin B_6 and that maize was a good source of vitamin B_6. Copping[15] also found that maize contains vitamin B_6. Walker and Wheeler[16] used 285 Gm. of maize daily per patient in producing human pellagra. Dann[4] reported that white or yellow maize meal contained vitamin B_6, and Birch and György[17] also concluded that vitamin B_6 is not identical with the pellagra-preventive vitamin. Fouts, Lepkovsky, Helmer and Jukes[5] successfully treated two cases of pellagra with a preparation free of vitamin B_6. Crystalline vitamin B_6 has been prepared in four different laboratories[17a] and work is now going forward on the determination of its chemical structure. It has been shown that the crystalline material will cure the specific dermatitis in rats.[17b]

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[4] Dann, W. J.: The Vitamin G Complex: I. The Nonidentity of Rat Dermatitis Due to Vitamin B_6 Deficiency and the Dermatitis of Human Pellagra, J. Nutrition **11:** 451 (May) 1936.

[5] Fouts, P. J.; Lepkovsky, Samuel; Helmer, O. M., and Jukes, T. H.: Successful Treatment of Human Pellagra with the "Filtrate-Factor," Proc. Soc. Exper. Biol. & Med. **35:** 245 (Nov.) 1936.

[6] Koehn, C. J., Jr., and Elvehjem, C. A.: Studies on Vitamin G (B_2) and Its Relation to Canine Black Tongue, J. Nutrition **11:** 67 (Jan.) 1936.

[7] Sebrell, W. H.; Hunt, D. J., and Onstott, R. H.: Lactoflavin in the Treatment of Canine Blacktongue, Pub. Health Rep. **52:** 235 (Feb. 26) 1937.

[8] Kisch, Franz: Vitamin B_2 (Lactoflavin in der Behandlung funikulärer Myelose bei perniziöser Anämie, Wien. med. Wchnschr. **87:** 194 (Feb. 13) 1937.

[9] Sebrell, W. H.: III. "Yellow Liver" of Dogs (Fatty Infiltration) Associated with Deficient Diets, Nat. Inst. Health Bull. 162, 1933.

[10] Sebrell, W. H.; Onstott, R. H., and Hunt, D. J.: The Treatment of Blacktongue with a Preparation Containing the "Filtrate Factor," and Evidence of Riboflavin Deficiency in Dogs, Pub. Health Rep. **52:** 427 (April 9) 1937.

[11] György, Paul: Vitamin B_2 and the Pellagra-like Dermatitis in Rats, Nature **133:** 498 (March 31) 1934.

[12] Chick, Harriette; Copping, Alice M., and Edgar, Constance E.: The Water-Soluble B-Vitamins: IV. The Components of Vitamin B_2, Biochem. J. **29:** 722 (No. 3) 1935.

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[13] Chick, Harriette, and Copping, Alice M.: The Composite Nature of the Water-Soluble Vitamin B: III. Dietary Factors in Addition to the Antineuritic Vitamin B_1 and the Antidermatitis Vitamin B_2, Biochem. J. **24**: 1764 (No. 6) 1930.

[14] Jukes, T. H.: Further Observations on the Assay, Distribution and Properties of the Filtrate Factor, J. Biol. Chem. **117**: 11 (Jan.) 1937.

[15] Copping, Alice M.: The Water-Soluble B Vitamins: VI. Flavin and Vitamin B_6 in Cereals, Biochem. J. **30**: 849 (May) 1936.

[16] Walker, N. P., and Wheeler, G. A.: Influence on Epilepsy of a Diet Low in the Pellagra-Preventive Factor, Pub. Health Rep. **46**: 851 (April 10) 1931.

[17] Birch, T. W., and György, Paul: Vitamin B_6 and Human Pellagra, J. Soc. Chem. Ind. **54**: 507 (May 24) 1935.

[17a] (*a*) Keresztesy, J. C., and Stevens, J. R.: Crystalline Vitamin B_6, Proc. Soc. Exper. Biol. & Med. **38**: 64 (Feb.) 1938. (*b*) Lepkovsky, Samuel: Crystalline Factor I, Science **87**: 169 (Feb. 18) 1938. György, Paul: Crystalline Vitamin B_6, J. Am. Chem. Soc. **60**: 983 (April) 1938. Kuhn, Richard, and Wendt, Gerhard: Ueber das aus Reiskleie und Hefe isolierte Adermin (Vitamin B_6), Ber. Deut. Chem. Gesell. **71**: 1118 (May 4) 1938.

[17b] Keresztesy and Stevens.[17a]

THE FILTRATE FACTOR OR CHICK PELLAGRA FACTOR

At the present time there is considerable evidence that the filtrate factor or chick pellagra factor is distinct from the pellagra-preventive factor. Fouts, Lepkovsky, Helmer and Jukes[5] successfully treated two cases of pellagra with a concentrate containing the filtrate factor prepared from rice bran. Koehn and Elvehjm[6] cured blacktongue with a liver extract which they state was found active for chicks. Sebrell, Onstott and Hunt[10] showed that another preparation containing the filtrate factor contained the blacktongue-preventive factor. Jukes[14] has assayed several foods for the filtrate factor and finds striking differences between the pellagra-preventive value of foods and their filtrate factor value. He finds that beef, whole corn meal and wheat germ are of the same order of potency in filtrate factor value, although corn meal is one of the poorest sources of the

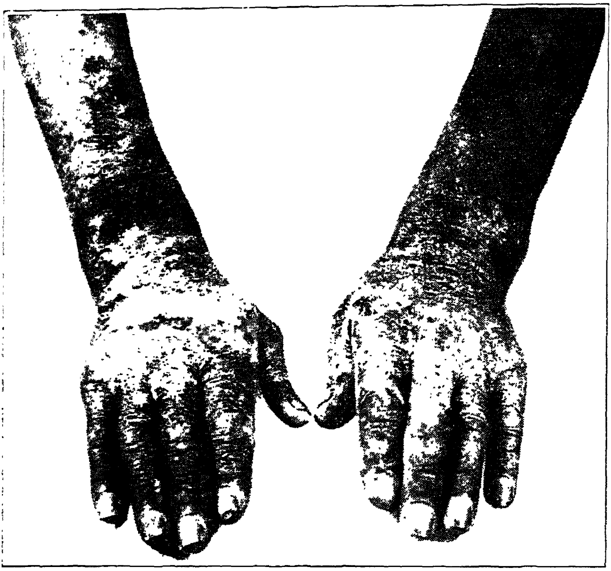

Fig. 1.—Characteristic dermatitis of the fingers, hands, wrists and forearms in pellagra. Reproduced by courtesy of Dr. T. D. Spies and the J. B. Lippincott Company, Philadelphia.

pellagra-preventive vitamin, while beef and wheat germ are two of the good sources of this vitamin. Dann and Subbarow[17c] have shown that nicotinic acid, which will cure pellagra, is without value in the prevention of chick dermatitis. This strongly suggests that the filtrate factor is distinct from the pellagra-preventive factor, but more evidence is needed before this question can be answered conclusively.

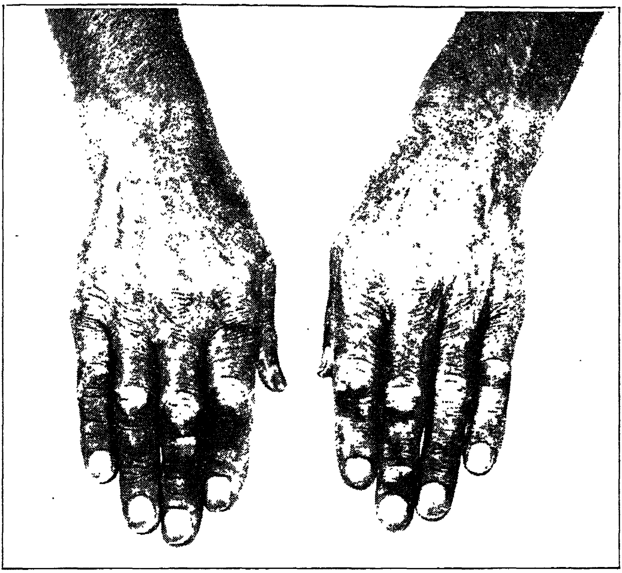

Fig. 2.—Appearance of the skin lesions 19 days later and after healing had set in. Reproduced by courtesy of Dr. T. D. Spies and the J. B. Lippincott Company, Philadelphia.

THE PELLAGRA-PREVENTIVE OR BLACKTONGUE-PREVENTIVE FACTOR

The term pellagra-preventive or blacktongue-preventive factor is applied to the substance originally postulated by Goldberger and Tanner[18] as necessary for

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17c. Dann, W. J., and Subbarow, Yellapragada: Differentiation of the Rat Dermatitis Factor and the Chick Dermatitis Factor from Nicotinic Acid, J. Nutrition **16**: 183 (Aug. 10) 1938.

18. Goldberger, Joseph, and Tanner, W. F.: A Study of the Treatment and Prevention of Pellagra: Experiments Showing the Value of Fresh Meat and of Milk, the Therapeutic Failure of Gelatin, and the Preventive Failure of Butter and of Cod Liver Oil, Pub. Health Rep. **39**: 87 (Jan. 18) 1924. Goldberger and Tanner.[24]

the prevention of human pellagra. There is no confusion with regard to the substance referred to when its definition is restricted to this meaning. Whether or not any of the other later identified fractions of the vitamin B complex are identical with this one is problematic, as indicated in the preceding discussion. Whether a deficiency in this factor will produce a symptom complex in rats or in any other experimental animals other than dogs remains to be demonstrated, and tests with pellagra or blacktongue are the only sound experimetal methods for work on the pellagra-preventive vitamin at the present time. Human experiments served a very useful purpose before a suitable experimental animal was available and yielded information of immense value which could have been obtained in no other way. Goldberger and Wheeler and their associates[19] conducted a long series of preventive tests on man. These experiments were conducted by adding the foodstuff to be tested to a known pellagra-producing diet and feeding this supplemented diet to a group of subjects. If pellagra occurred in the group, it was concluded that the food added as a supplement contained little or none of the pellagra-preventive factor in the quantity given. If no pellagra occurred in the group during one year's observation, it was concluded that the food contained the pellagra-preventive factor. These experiments were supplemented by

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19. These tests are described by:

Goldberger, Joseph; Wheeler, G. A.; Lillie, R. D., and Rogers, L. M.: A Further Study of Butter, Fresh Beef and Yeast as Pellagra Preventives with Consideration of the Relation of Factor P-P of Pellagra (and Black Tongue of Dogs) to Vitamin B, Pub. Health Rep. **41**: 297 (Feb. 19) 1926.

Goldberger, Joseph, and Wheeler, G. A.: A Study of the Pellagra-Preventive Action of the Tomato, Carrot and Rutabaga Turnip, ibid. **42**: 1299 (May 13) 1927.

A Study of the Pellagra-Preventive Action of the Cowpea (Vigna Sinensis) and of Commercial Wheat Germ, ibid. **42**: 2383 (Sept. 30) 1927.

A Study of the Pellagra-Preventive Action of Canned Salmon, ibid. **44**: 2769 (Nov. 15) 1929.

Wheeler, G. A.: The Pellagra-Preventive Value of Canned Spinach, Canned Turnip Greens, Mature Onions, and Canned Green Beans, ibid. **46**: 2663 (Nov. 6) 1931. Wheeler.[25]

Wheeler, G. A., and Hunt, D. J.: The Pellagra-Preventive Value of Green Cabbage, Collards, Mustard Greens, and Kale, ibid. **48**: 754 (June 30) 1933.

The Pellagra-Preventive Value of Green Onions, Lettuce Leaves, Pork Shoulder and Peanut Meal, ibid. **49**: 732 (June 22) 1934.

Goldberger and Tanner.[16]

tests with blacktongue in dogs until it was demonstrated that the two diseases were analogous.[20]

The evidence that blacktongue of dogs and human pellagra are analogous conditions produced by a deficiency in the same dietary factor is as follows: (*a*) Every food or substance that has been tested in the prevention or treatment of both pellagra and blacktongue and found to be of value in one disease has been found to be of value also in the other; (*b*) every food or substance tested in both diseases that has proved worthless in the prevention or treatment of one has also proved worthless in the prevention or treatment of the other; (*c*) the symptomatology is similar in that in both diseases there is a severe stomatitis, a diarrhea, a slight degree of secondary anemia and a dermatitis of the scrotum; finally, the gross and microscopic pathologic appearances are similar.

Many experimental conditions in rats have been described as pellagra-like,[21] but there is no evidence that a deficiency in the pellagra-preventive factor causes a dermatitis in rats. In 1934 Sebrell[22] pointed out that what was being called vitamin G and assayed by growth of rats did not necessarily bear any relationship to the pellagra-preventive value of the material.

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[20] These experiments are described by:

Goldberger, Joseph, and Wheeler, G. A.: Experimental Black Tongue of Dogs and Its Relation to Pellagra, Pub. Health Rep. **43:** 172 (Jan. 27) 1928.

Goldberger, Joseph; Wheeler, G. A.; Lillie, R. D., and Rogers, L. M.: A Further Study of Experimental Blacktongue with Special Reference to the Blacktongue Preventive in Yeast, ibid. **43:** 657 (March 23) 1928.

A Study of the Blacktongue-Preventive Action of Sixteen Foodstuffs, with Special Reference to the Identity of Blacktongue of Dogs and Pellagra of Man, ibid. **43:** 1385 (June 8) 1928.

Goldberger, Joseph; Wheeler, G. A.; Rogers, L. M., and Sebrell, W. H.: A Study of the Blacktongue-Preventive Value of Leached Commercial Casein, Together with a Test of the Blacktongue-Preventive Action of a High Protein Diet, ibid. **45:** 273 (Feb. 7) 1930.

A Study of the Blacktongue-Preventive Value of Lard, Salt Pork, Dried Green Peas and Canned Haddock, ibid. **45:** 1297 (June 6) 1930.

Sebrell, W. H.; Wheeler, G. A., and Hunt, D. J.: The Blacktongue-Preventive Value of Seven Foodstuffs, ibid. **50:** 1333 (Sept. 27) 1935

Wheeler, G. A., and Sebrell, W. H.: The Blacktongue (Canine Pellagra) Preventive Value of Fifteen Foodstuffs, Nat. Inst. Health Bull. 162, 1933.

[21] Burr, G. O., and Burr, Mildred M.: On the Nature and Rôle of the Fatty Acids Essential in Nutrition, J. Biol. Chem. **86:** 587 (April) 1930. Salmon, W. D.; Hayes, I. M., and Guerrant, N. B.: Etiology of Dermatitis of Experimental Pellagra in Rats, J. Infect. Dis. **43:** 426 (Nov.) 1928. Parsons, Helen T.: The Physiological Effects of Diets Rich in Egg White, J. Biol. Chem. **90:** 351 (Jan.) 1931.

[22] Sebrell, W. H.: Pellagra, Virginia M. Monthly **61:** 136 (June) 1934.

Since practically all of the vitamin G determinations of foods have been made by rat growth experiments and the results frequently expressed in terms of Sherman-Bourquin units, which is a measure of their riboflavin content, the tables of the vitamin G content of foods in the various textbooks indicate the riboflavin, and not the pellagra-preventive vitamin, content of foods. These tables therefore should not be used for the selection of foods in the treatment of pellagra. The determinations of the pellagra-preventive value of foods by Goldberger and his associates give the only data that can be safely used in the treatment of pellagra. These data are summarized in the accompanying table.

MULTIPLE DEFICIENCIES AND PELLAGRA

It has been suggested that a deficiency in more than one factor is involved in pellagra and that pellagra as it is now recognized actually consists of two or more different conditions.

In 1918 Goldberger, Wheeler and Sydenstricker[23] suggested that two separate dietary factors may be concerned in pellagra. They said: "The diagnosis of pellagra was restricted to cases presenting a definite, bilaterally, symmetrical eruption. It is suggested that, so defined, pellagra includes at least two commonly associated, etiologically distinct, though closely related, syndromes." Goldberger,[24] in his experiments with casein, made particular mention of the fact that the patients had symptoms diagnosed as pellagra *sine* pellagra, although they never developed skin lesions. In 1933 Wheeler[25] discussed at some length the possibility of there being two different conditions. Wright[26] described a condition in Sierra Leone characterized by glossitis and skin lesions, especially at the mucocutaneous junction, associated with or followed by dis-

944 (Sept. 21) 1918.

[24] Goldberger, Joseph, and Tanner, W. F.: A Study of the Pellagra-Preventive Action of Dried Beans, Casein, Dried Milk and Brewers' Yeast, with a Consideration of the Essential Preventive Factors Involved, Pub. Health Rep. **40:** 54 (Jan. 9) 1925.

[25] Wheeler, G. A.: The Pellagra-Preventive Value of Autoclaved Dried Yeast, Canned Flaked Haddock, and Canned Green Peas, Pub. Health Rep. **48:** 67 (Jan. 20) 1933.

[26] Wright, E. J.: The A and B Avitaminosis Disease of Sierra Leone, from Leitch, J. Neil: Dietetics in Warm Climates, London, Harrison & Sons, Ltd., 1930, p. 367.

orders of the nervous system, which was cured by the use of cod liver oil and yeast. Stannus[27] holds that such lesions are manifestations of pellagra. Fitzgerald[28] described an outbreak of exfoliative glossitis with ulceration at the angles of the mouth, salivation, gastrointestinal symptoms and diarrhea, which occurred in an Assam prison. Adding 1 ounce of yeast daily to the diet was found to be of value in treatment. Moore[29] described a syndrome of loss of visual acuity, sore tongue, sore mouth, white patches at the edges of the lips, and scrotal or vulval rash in school children in Nigeria, which he believes due to food. Landor and Pallister[30] have studied a condition in the prisons of Singapore and Johore, characterized by glossitis, lesions in the angles of the lips, an eczematous condition of the scrotum and, in the later stages, nerve symptoms suggestive of subacute combined degeneration of the cord nearly always accompanied by dimness of vision. Patients with the glossitis and eczema of the scrotum were successfully treated with fresh brewers' yeast, autoclaved yeast, autoclaved marmite, or liver. Cod liver oil and orange juice were without value in treatment. These authors note particularly that the typical dermatitis of pellagra was not present. They state that "there is much resemblance between the disease we have described and pellagra, but we feel that it would be incorrect to use the term pellagra for it and prefer to consider it avitaminosis B_2." Aykroyd and Krishnan[31] describe a stomatitis in children in South India due to a deficiency of one or more factors in the vitamin B_2 complex. They did not observe any involvement of the central nervous system and nothing suggesting pellagrous dermatitis or diarrhea was present in their cases. The disease is curable by fresh, unheated or autoclaved yeast, liver and skim milk. The suggestion is made that the condition may be a manifestation of flavin deficiency.

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27. Stannus, H. S.: Deficiency Diseases in Sierre Leone and Pellagra, Tr. Roy. Soc. Trop. Med. & Hyg. **23:** 627 (April) 1930.

28. Fitzgerald, G. H.: An Outbreak of Exfoliative Glossitis in an Assam Jail, Indian M. Gaz. **67:** 556 (Oct.) 1932.

29. Moore, D. G. F.: Retrobulbar Neuritis and Partial Optic Atrophy as Sequelae of Avitaminosis, Ann. Trop. Med. & Parasit. **28:** 295 (Oct.) 1934.

30. Landor, J. V., and Pallister, R. A.: Avitaminosis B_2, Tr. Roy. Soc. Trop. Med. & Hyg. **29:** 121 (July) 1935.

31. Aykroyd, W. R., and Krishnan, B. G.: Stomatitis Due to Vitamin B_2 Deficiency, Indian J. M. Research **24:** 411 (Oct.) 1936.

Pellagra-Preventive Value of Foods *

Food	Quantity Used, Gm.	Pellagra-Preventive Value
Cereals		
Corn-meal, whole, white	450	None
Cornstarch	366	None
Oats, rolled	400	None
Rye meal	400	None
Wheat germ (ether extracted)	150	Good
Wheat, whole	400	Slight
Dairy Products		
Butter	135	Slight
Casein, leached	85	Slight
Egg, yolk (dried)	100	Fair
Milk:		
Buttermilk	1,200	Good
Evaporated (canned)	...†	Fair
Skim, dried	105	Fair
fresh	...‡	Fair
Fruits		
Apples, evaporated	250	None
Prunes, dried	250	None
Meats and Fish		
Beef:		
Corned (canned)	200	Good
Fresh, lean	200	Good
Chicken (canned)	325	Good
Haddock (canned)	340	Fair
Liver, pork (dried)	64	Good
Pork:		
Salt, fat	153	None
Shoulder, lean	200	Good
Rabbit	184	Good
Salmon (canned)	168	Good
Oils and Fats		
Butter (see dairy products)		
Cod liver oil	128	None
Cottonseed oil	110	None
Lard	110	None
Vegetables		
Beans		
Green, stringless (canned)	550	Slight
Kidney, red	360	Fair
Navy	360	None
Soya	360	Fair
Cabbage, green (canned)	482	Fair
Carrots	450	Slight
Collards (canned)	482	Good
Cowpeas	178	Fair
Kale (canned)	534	Good
Lettuce, cos (canned)	516	Slight
Mustard greens (canned)	533	Fair
Onions:		
Green (canned)	502	Slight
Mature	525	None
Peas:		
Green (canned)	450	Good
Green (dried)	360	Fair
Potatoes:		
Irish	450	None
Sweet	450	None
Spinach (canned)	482	Fair
Tomato, juice from canned	1,200	Good
Turnip greens (canned)	482	Good
Turnips, rutabaga	453	Slight

Pellagra-Preventive Value of Foods—Continued*

Food	Quantity Used, Gm.	Pellagra-Preventive Value
Miscellaneous		
Cottonseed meal	200	Slight
Gelatin	83	None
Liver extract (Minot's 343)	...§	Fair
Peanut meal	200	Good
Rice polishings	400	Good
Yeast:		
Bakers', dried	30	Good
Bakers', dried, autoclaved	60	Good
Brewers', dried	30	Good
Yeast vitamin powder	15	Good

* Adapted from "Table Showing the Pellagra-Preventive Value of Various Foods, Pub. Health Rep. **49**: 754 (June 29) 1934.

† 15 cc. per kilogram of body weight.

‡ 30 cc. per kilogram of body weight.

§ Equivalent to 100 Gm. of liver.

This table is intended primarily for use in the treatment and prevention of pellagra, and only those foods are included which have been tested under controlled conditions in either human beings or dogs or both. In the absence of a quantitative method of assaying the pellagra-preventive vitamin, only the most general terms can be used to designate the pellagra-preventive value of a food. In order to make a division into groups which will be of practical value without being unwarrantedly exact, the words "good," "fair," "slight" and "none" have been selected. The quantity used must be kept in mind in each instance, since smaller amounts than those indicated would in all probability have less value.

"Good" signifies that the indicated quantity of food contained enough of the pellagra-preventive factor to prevent the disease. This is the most valuable class of foods in the prevention and treatment of pellagra.

"Fair" signifies that the indicated quantity of food showed appreciable and in some instances considerable pellagra-preventive value, but one or more of the experimental subjects developed the disease, usually after considerable delay. Thus, a food under this heading contains enough of the vitamin to be of value but should not be relied on alone in the treatment and prevention of the disease. The principal value of these foods lies in the variety of items afforded as adjuncts to the good sources of the preventive factor.

"Slight" signifies that the indicated quantity of food, although failing to prevent the disease, caused a slight delay in onset. Practically, this group may be disregarded in the treatment and prevention of pellagra.

"None" signifies that, in the quantity used, the results of the experiments indicate that the food contains either none of the preventive factor or such a small amount that it may be regarded, for practical purposes, as being entirely without value in the treatment and prevention of pellagra.

Stannus[32] is of the opinion that these conditions are pellagra. Ruffin and Smith,[33] as a result of their work with liver fractions, think that there is a deficiency in more than one substance in pellagra, and Sydenstricker and Thomas [34] have postulated an intrinsic and an extrinsic factor in pellagra. Spies and Aring [34a] have reported that the peripheral neuritis which occurs in some cases of pellagra responds to treatment with crystalline vitamin B_1.

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[32] Stannus, H. S.: Pellagra and Pellagra-like Conditions in Warm Climates: III, Trop. Dis. Bull. **33**: 885 (Dec.) 1936.

[33] Ruffin, J. M.; Persons, E. R.; Harvey, H. I., and Smith, D. T.: Evidence for the Existence of Two Factors Necessary for the Successful Treatment of Pellagra or Experimental Canine Black Tongue, J. Clin. Investigation **16**: 663 (July) 1937.

In view of the uncertainty surrounding both the number of factors in the vitamin B complex and their physiologic action. it appears not unlikely that some of the symptoms hitherto assigned to the pellagra syndrome may be manifestations of deficiencies in factors other than the one responsible for the typical attack with characteristic dermal lesions for which the names "pellagra" and "pellagra-preventive factor" obviously should be retained. However, since the factors of the vitamin B complex. fortunately, are closely associated in many foodstuffs, these questions become of serious import in treatment only when one begins to treat pellagra with purified vitamin fractions. This has been pointed out recently by Sebrell, Onstott and Hunt [10] in connection with a purified rice bran fraction which contained enough of the pellagra-preventive vitamin to cure blacktongue, but the experimental animals died as a result of flavin deficiency. The fact that both the gastro-intestinal and dermal lesions of pellagra clear up under treatment with nicotinic acid strongly indicates that only one factor is involved in these lesions. Further experimentation with nicotinic acid should reveal whether the varied clinical changes which in the past have been called pellagra are the result of a deficiency in one substance.

SUNLIGHT AS A FACTOR IN PELLAGRA

It has been recognized since the very earliest studies on pellagra that sunlight has a deleterious effect on pellagrins. Frapolli [35] in 1771, Albera [36] in 1784, Gherardini [37] in 1792 and Calderini [38] in 1844, among other early students of the disease, refer to the effect of sunlight in pellagra. Larriba [39] in 1881 thought that the intensity of the erythema depends directly on an internal factor and on the action of sunlight. The literature is extensively quoted by Smith and Ruffin,[40] who, since the demonstration that a deficient diet is the primary cause of pellagra, have reopened the question of the secondary importance of sunlight. These authors, by keeping pellagrins on a deficient diet, have succeeded in causing the dermatitis to reappear in thirteen out of thirty-five patients by exposing them to the sun. Fouts and Zerfas [41] report the reappearance of the dermatitis by exposure to sunlight in a case in which the dermatitis was clearing rapidly and another case in which the dermatitis appeared on the hands after a fifteen minute exposure to the sun. Bigland [42] states that he was unable to produce skin changes by exposure to the sun, although he thinks that the sun's rays play an important part in the production of the pellagrous dermatitis. Spies [43] produced the dermatitis of pellagra in four volunteers who were kept out of direct sunlight while receiving a pellagra-producing diet, and in eight cases the dermatitis healed in spite of long, daily exposure to direct sunlight. In ten patients there was no effect from exposure to radiation from a quartz mercury vapor arc lamp. Crutchfield [44] saw no appreciable effect on treating ten patients with very small amounts of ultraviolet rays and thinks that trauma is a more important factor than sunlight. Niles [45] states that exposure to the sun or to radiant heat from a stove or furnace will bring out an erythema in a pellagrin. Bass [46] reports the development of pellagrous dermatitis from exposure to heat from a stove, and Smith and Ruffin [40] report three cases in which severe symptoms followed exposure to a red-

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[34] 34. Sydenstricker, V. P., and Thomas, J. W.: Some Factors in the Etiology of Pellagra, South. M. J. **30:** 14 (Jan.) 1937.

[34a] 34a. Spies, T. D., and Aring, C. D.: The Effect of Vitamin B_1 on the Peripheral Neuritis of Pellagra, J. A. M. A. **110:** 1081 (April 2) 1938.

[35] 35. Frapolli, Francisci: Animadversiones in Morbum vulgo Pelagram, Milan, 1771.

[36] 36. Albera, G. M.: Trattato teorico practico delle malattie dell' insolato di primavera volgarmente dette della pellagra, Del medico Fisico, per Gaetano Motta e Giorgio Pedemonti, 1784.

[37] 37 Gherardini, Michaeli: Geschichte des Pellagra aus dem Italianischen, Lemgo im Berlage der Meyerschen Buchhandlung, 1792.

[38] 38. Calderini, Carlo-Gallo: Rapport intorno ai pellagrosi d'ambo i sessi stati assoggettati nell' Ospedale Maggiore di Milano alla cura balnearnia, nella estate dell'anno 1843, Annali Universali di Medicina **110:** 26, Milan, 1844.

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39. Larriba, Don Gregorio Calmarza: La Pellagra, Calatayud, Imprenta del diario, 1881.
40. Smith, D. T., and Ruffin, J. M.: Effect of Sunlight on the Clinical Manifestations of Pellagra, Arch. Int. Med. **59:** 631 (April) 1937.
41. Fouts, P. J., and Zerfas, L. G.: Pellagra in Indiana and Its Treatment, J. Indiana M. A. **27:** 196 (May) 1934.
42. Bigland, A. D.: The Pellagra Outbreak in Egypt: 1. Pellagra Among Ottoman Prisoners of War, Lancet **1:** 947 (May 1) 1920.
43. Spies, T. D.: Relationship of Pellagrous Dermatitis to Sunlight, Arch. Int. Med. **56:** 920 (Nov.) 1935.
44. Crutchfield, E. D.: Pellagra, with Special Reference to the Skin and Mucous Membrane, Arch. Dermat. & Syph. **17:** 650 (May) 1928.
45. Niles, G. M.: The Treatment of Pellagra, J. A. M. A. **62:** 285 (Jan. 24) 1914.
46. Bass, C. C.: Pellagra, M. Clin. North America **12:** 1181 (March) 1929.

hot stove in one instance, a brush fire in another, and an electric heater in a third. Bass [47] notes that exposure to heat, pressure, trauma and irritating chemicals, as well as sunlight, may cause the skin lesions. Stannus [47a] states that "to those who have studied pellagra closely it has become obvious that there is a tendency for the exanthem to occur in any area of the skin open to trauma of any kind, using the word in its widest sense."

It appears, therefore, on the basis of the evidence now available, that the action of sunlight in pellagra is to be regarded as that of an irritant, and pellagrins should be protected from exposure to sunlight and skin irritants of all kinds. It should be recognized that this is a point of relatively minor importance in treatment and that recovery cannot be expected unless the proper dietary corrections are made.

PORPHYRINS AND PELLAGRA

Masso [48] has reported a moderate elimination of uroporphyrin and a copious elimination of coproporphyrin in two cases of pellagra. Bassi [49] and Ellinger and Dojmi [50] reported porphyrinuria in pellagra, and Sydenstricker and Thomas [34] suggest that the dermatitis in pellagra is due to photosensitization by hematoporphyrin. Beckh, Ellinger and Spies [50a] obtained inconclusive results in a study of porphyrinuria in pellagra, but Spies, Sasaki and Cross [50b] report increased urinary porphyrin in twenty pellagrins, which, in sixteen cases, returned to normal following treatment with yeast, liver extract or nicotinic acid. They conclude that increased porphyrinuria is an integral part of the pellagra syndrome. However, Spies, Aring, Gelperin and Bean [50c] recently have reported that mesobiliviolin gives a positive test with the color reaction used by Spies,

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[47] Bass, C. C.: Pellagra. M. Clin. North America **9:** 869 (Jan.) 19-6.

[47a] Stannus, H. S.: Pellagra and Pellagra-like Conditions in Warm Climates: II, Trop. Dis. Bull. **33:** 815 (Nov.) 1936.

[48] Masso, Mario: Sensibilizzazione alla luce e porfirine nei riguardi di [illegible]

[50] Ellinger, P., and Dojmi, L.: Note on the Presence of Porphyrin in the Urine of Pellagra Patients, J. Soc. Chem. Ind. **54:** 507, 1935.

[50a] Beckh, W.; Ellinger, P., and Spies, T. D.: Quart. J. Med. **6:** 305 (July) 1937.

[50b] Spies, T. D.; Saski, Yasuo, and Cross, Esther: A Note on the Relationship of Porphyrinuria to Human Pellagra, South. M. J. **31:** 483 (May) 1938.

[50c] Spies, T. D.; Aring, C. D.; Gelperin, Jules, and Bean, W. B.: The Mental Symptoms of Pellagra. Their Relief with Nicotinic Acid, Am. J. M. Sc. **196:** 461 (Oct.) 1938.

Sasaki and Cross[50b] for porphyrin determinations. They also note a seasonal variation in the occurrence of strongly positive tests. Dobriner, Strain and Localio[51] found, in a case of alcoholic pellagra, both a quantitative and qualitative increase in porphyrin excretion, which decreased on treatment with yeast extract and liver extract.

The constancy of the occurrence and the fundamental importance of porphyrinuria in pellagra are, therefore, still uncertain.

LIVER EXTRACTS, ANTIPERNICIOUS ANEMIA FACTORS AND PELLAGRA

The first report on the probable value of liver extracts in pellagra is that by Goldberger and Sebrell,[52] who tested Liver Extract-Lilly in blacktongue and found that in the quantity used it had a therapeutic and preventive effect. Rhoads and Miller[53] produced a condition in dogs which they described as chronic blacktongue with anemia, which they were able to prevent by feeding 4 Gm. of Liver Extract-Lilly daily. They failed to demonstrate any improvement, however, on treating the condition with large doses of liver extract parenterally, and the daily intramuscular injection of the liver extract from 50 Gm. of whole liver failed to prevent the anemia and glossitis. The first report on the use of liver extracts in pellagra is that of Ramsdell and Magness[54] in 1933. These workers treated twenty-two cases by the intramuscular injection daily of 2 cc. of Liver Extract-Lilly, at the same time placing the patients on the general hospital diet. They state that the results were spectacular in that the appetite returned in from three to seven days and there was rapid clinical improvement in all cases. Ramsdell[55] later added three cases to the series. Ruffin and Smith,[56] however, saw little or no improvement in five patients kept on a diet deficient in the pellagra-preventive vitamin and given daily doses of 5 cc. of Liver Extract-Lilly intramuscularly. The treatment of four of these patients was changed to 90 cc. of an aqueous extract of liver daily, by mouth, with rapid recovery. They treated ten additional patients with 90 cc. of the aqueous liver extract daily, by mouth, with satisfactory results. These authors attribute the successful results of Ramsdell and Magness to the good diet used by the latter. Spies[57] put sixteen patients on a diet deficient in the pellagra-preventive factor and gave ten of these 80 cc. of liver extract intravenously, and six from 24 to 30 cc. of liver extract intramuscularly. All the patients quickly responded. The failure of the treatment of Ruffin and Smith possibly may be explained by the smaller doses used by them or by the fact that a different liver preparation was used. Fouts and Zerfas[41] treated four cases of pellagra with liver extract by mouth and intravenously, with satisfactory results. Spies[58] recommends the oral administration of from 75 to 100 Gm. of liver extract daily, or from three to five intravenous doses of 20 cc. each in the treatment of severe cases of pellagra. Ruffin and Smith[59] report rapid and complete recovery of twenty out of twenty-one cases of pellagra from the use of a daily dose of a liquid liver extract (Valentine) derived from 675 Gm. of liver (90 cc.) for from five to twenty-five days; with Liver Extract-Lilly intramuscularly in doses derived from 15 to 20 Gm. of liver, only two of nine patients recovered; with Lederle's parenteral liver extract in doses derived from 100 Gm. of liver, none of eight recovered, and with Parke, Davis's liver extract intravenously, one of three recovered. These were treated, however, for only one and two days with doses derived from 200, 250 and 500 Gm. of liver. These results do not agree with the experience of Spies,[58] who thinks that the treatment of severe cases is most successful when large doses of liver extract are used parenterally.

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[51] Dobriner, Konrad; Strain, W. H., and Localio, S. A.: The Excretion of Porphyrin in Pellagra, Proc. Soc. Exper. Biol. & Med. **38:** 548 (June) 11938.

[52] Goldberger, Joseph, and Sebrell, W. H.: The Blacktongue-Preventive Value of Minot's Liver Extract, Pub. Health Rep. **45:** 3064 (Dec. 12) 1930.

[53] Rhoads, C. P., and Miller, D. K.: Production in Dogs of Chronic Blacktongue with Anemia, J. Exper. Med. **58:** 585 (Nov.) 1933.

[54] Ramsdell, R. L., and Magness, W. H.: Parenteral Liver Extract Therapy in the Treatment of Pellagra: A Preliminary Report, Am. J. M. Sc. **185:** 568, 1933.

[55] Ramsdell, R. L.: The Treatment of Pellagra: Results Obtained by Liver Extract Used Parenterally in Twenty-Five Cases, Texas State J. Med. **29:** 211 (July) 1933.

[56] Ruffin, J. M., and Smith, D. T.: The Treatment of Pellagra with Certain Preparations of Liver, Am. J. M. Sc. **187:** 512 (April) 1934.

The evidence appears to warrant the therapeutic use of liver extracts, both orally and parenterally, in severe

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57. Spies, T. D.: Observations on the Treatment of Pellagra, J. Clin. Investigation **13**: 807 (Sept.) 1934.

58. Spies, T. D.: The Treatment of Pellagra, J. A. M. A. **104**: 1377 (April 20) 1935.

59. Ruffin, J. M., and Smith, D. T.: A Clinical Evaluation of the Potency of Various Extracts of Liver in the Treatment of Pellagra, South. M. J. **30**: 4 (Jan.) 1937.

cases of pellagra. It should be borne in mind that liver extracts are prepared and assayed for use in the treatment of pernicious anemia and that their value in pernicious anemia does not necessarily bear any relationship to their value in pellagra. Therefore, large doses are essential. Their principal value would appear to be as an adjunct to nicotinic acid, yeast and the dietary treatment, and as a means of parenteral administration of the vitamin when severe vomiting or diarrhea makes the retention and utilization of food by mouth difficult or impossible. They should be used in very liberal doses in conjunction with yeast, milk, lean meat, and all the other foods rich in the pellagra-preventive vitamin.

The possibility that the pellagra-preventive vitamin and the extrinsic antipernicious anemia factor may be identical or related substances has attracted some attention. Before riboflavin was differentiated from the other factors in the vitamin B complex, Strauss and Castle[60] suggested that the two factors were identical, since they found substances containing the antipernicious anemia factor also to contain the rat growth promoting vitamin G. Strauss and Castle[61] obtained a response in pernicious anemia with an incubated mixture of normal gastric juice and an autolyzed yeast preparation, and Miller and Rhoads,[62] using a modified blacktongue-producing diet, described a symptom complex in swine characterized by lesions of the buccal mucosa, achlorhydria, and anemia associated with a loss of the anti-pernicious anemia activity of the gastric secretion. Spies[58] showed that desiccated hog stomach (Ventriculin) has therapeutic value in pellagra, and Spies and Grant[63] also suggested that the antianemic factor may be vitamin G or some closely related factor. Later, Spies, Payne and Chinn[64] treated cases of pernicious anemia with autoclaved yeast incubated with normal human gastric juice and treated pellagrins with the same lot of autoclaved yeast. There was no remission of the pernicious anemia, although the pellagra was cured. Wills [65] investigated the hematopoietic effect of several preparations containing the B vitamins, and Wills and Naish [66] found no response in pernicious anemia with an egg white preparation rich in vitamin B_2. Davidson,[67] from an investigation of autolyzed yeast products (marmite) in sixteen cases of pernicious anemia, concludes that the hematopoietic factor in marmite, liver and liver extract is not vitamin B_2. Salah [68] found that the achylic gastric juice from five pellagrins contained the hematopoietic principle as determined by the rat reticulocyte response and concludes that the extrinsic hematopoietic principle is different from the pellagra-preventive factor. Stare and Thompson [69] were unable to obtain a hematopoietic response in pernicious anemia with purified hepatoflavin. Subbarrow, Jacobson and Fiske [70] have isolated a fraction from liver which they find both reticulocytogenic in the guinea-pig and therapeutically potent in blacktongue, although the variations in the response of pellagrins to various liver extracts reported by Ruffin and Smith [59] suggest that the two substances are different. The evidence therefore favors the view that the pellagra-preventive factor and the extrinsic anti-pernicious anemia factor are different substances. Further work on the identification of the factors involved in both diseases is necessary before conclusions can be drawn with regard to the entire lack of relationship between the etiologic factors in the two diseases.

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[60] Strauss, M. B., and Castle, W. B.: The Nature of the Extrinsic Factor of the Deficiency State in Pernicious Anemia and in Related Macrocytic Anemias: Activation of Yeast Derivatives with Normal Human Gastric Juice, New England J. Med. **207**: 55 (July 14) 1932.

[61] Strauss, M. B., and Castle, W. B.: The Extrinsic (Deficiency) Factor in Pernicious and Related Anaemias, Lancet **2**: 111 (July 16) 1932.

[62] Miller, D. K., and Rhoads, C. P.: Experimental Production of Loss of Hematopoietic Elements of Gastric Secretion and of Liver in Swine with Achlorhydria and Anemia, J. Clin. Investigation **14**: 153 (March) 1935.

[63] Spies, T. D., and Grant, Jean: An Experimental Study of a So-Called "Pellagra-Producing" Diet, Am. J. Physiol. **104**: 18 (April) 1933.

[64] Spies, T. D.; Payne, Warren, and Chinn, A. B.: A Note on the Relationship of Pellagra to Pernicious Anemia, Proc. Soc. Exper. Biol. & Med. **32**: 328 (Nov.) 1934.

YEAST IN PELLAGRA

Yeast was introduced in the treatment of pellagra by Goldberger and Tanner,[24] who used 1 Gm. of dried yeast per kilogram of body weight in treating the disease. Their experience led them to believe that a smaller dose was sufficient, particularly when combined with proper dietary treatment, and Goldberger, Wheeler and Tanner [71] finally recommended a daily dose of between 15 and 30 Gm. Walker and Wheeler [16] cured experimental pellagra with 30 Gm. of dried powdered yeast daily, while the patients remained on the deficient diet, and Wheeler [25] prevented pellagra with a daily dose of 60 Gm. of autoclaved, dried bakers' yeast. Spies, Chinn and McLester [72] recently have used even larger doses in treatment. No evidence of deleterious effects from overdosage with pure dried yeast have been reported and it is essential that very large doses be given if treatment is to be successful. It becomes obvious that the use of moist yeast cakes or compressed yeast tablets is out of the question, since a moderate daily dose of 10 grain tablets would be 100 tablets. Therefore the only practical method of administration is in the form of pure dried powdered yeast, which must be given in terms of tablespoonfuls. Anything less than this represents insufficient treatment and may lead to failure to secure satisfactory results. Yeast may be conveniently administered stirred into milk, tomato juice and table syrup. A bouillon may be made with warm water and salt, it may be sprinkled on cooked cereals, and Spies [41] has given it successfully in eggnogs.

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[65] Wills, Lucy: The Nature of the Hemopoietic Factor in Marmite, Lancet **1**: 1283 (June 17) 1933.

[66] Wills, Lucy, and Naish, Alice: A Case of Pernicious Anaemia Treated with Vitamin B_2 from Egg White, Lancet **1**: 1286 (June 17) 1933.

[67] Davidson, L. S. P.: Autolysed Yeast Products in the Treatment of Anemia, Brit. M. J. **2**: 481 (Sept. 9) 1933.

Anemia, Proc. Soc. Exper. Biol. & Med. **33**: 64 (Oct.) 1935.

Pig

Black Tongue, New England J. Med. **212**: 663 (April 11) 1935.

NICOTINIC ACID AND PELLAGRA

Recent developments have indicated that nicotinic acid may prove to be a valuable therapeutic agent in the treatment of pellagra. Elvehjem, Madden, Strong and Woolley [73] have reported that nicotinic acid and nicotinic acid amide will cure blacktongue of dogs. Street and Cowgill [74] noted beneficial effects in two dogs with chronic blacktongue, and Chick, Macrae, Martin and Martin [75] cured a similar condition in swine with nicotinic acid. Sebrell, Onstott, Fraser and Daft[76] have confirmed Elvehjem's results and find that 6 mg. of nicotinic acid twice a week will prevent the disease in dogs on a blacktongue-producing diet during an experimental period of six months. Elvehjem, Madden, Strong and Woolley[73] suggested clinical trial of their material in human pellagra. This was also suggested editorially in THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION.[77] An editorial in the *British Medical Journal*[78] has suggested the trial of trigonellin, the methylbetaine of nicotinic acid.

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[71] Goldberger, Joseph; Wheeler, G. A., and Tanner, W. F.: Yeast in the Treatment of Pellagra and Black Tongue: A Note on Dosage and Mode of Administration, Pub. Health Rep. **40:** 927 (May 8) 1925.

[72] Spies, T. D., Chinn, Austin, and McLester, J. B.: Treatment of Endemic Pellagra, South. M. J. **30:** 18 (Jan.) 1937.

[73] Elvehjem, C. A.; Madden, R. J.; Strong, F. M., and Woolley, D. W.: Relation of Nicotinic Acid and Nicotinic Acid Amide to Canine Black Tongue, J. Am. Chem. Soc. **59:** 1767 (Sept.) 1937.

[74] Street, H. R., and Cowgill, G. R.: The Cure of Canine Blacktongue with Nicotinic Acid, Proc. Soc. Exper. Biol. & Med. **37:** 547 (Dec.) 1937.

[75] Chick, Harriette; Macrae, T. F.; Martin, A. J. P., and Martin, C. J.: Curative Action of Nicotinic Acid on Pigs Suffering from the Effects of a Diet Consisting Largely of Maize, Biochem. J. **32:** 10 (Jan.) 1938.

Spies, Cooper and Blankenhorn[79] noted improvement in the mucous membrane lesions in four pellagrins within twelve hours after administering nicotinic acid. Fouts, Helmer, Lepkovsky and Jukes[80] reported that, following the administration of 500 mg. of nicotinic acid daily to three patients and 1 Gm. daily to one patient with pellagra, improvement was as satisfactory as that obtained by administering liver filtrate, except for an increase in the time required for complete disappearance of the dermatitis.

Harris[81] found that one-third Gm. of nicotinic acid daily hastened subsidence of erythema in five cases of pellagra. He has mentioned the possibility that nicotinic acid may not be the sole major deficiency in some pellagra-producing diets.

Spies[82] observed beneficial effects in fifteen cases and has noted that increased porphyrinuria associated with pellagra disappeared. He recommends the daily administration of 500 mg. of nicotinic acid in 100 mg. doses by mouth or 40 to 80 mg. in sterile physiologic solution of sodium chloride in 10 to 20 mg. doses. He also recommends that all patients be given a well balanced diet when nicotinic acid is used as a supplement.

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76. Sebrell, W. H.; Onstott, R. H.; Fraser, H. F., and Daft, F. S.: Read before the American Institute of Nutrition, March 30, 1938.

77. Relation of Nicotinic Acid to Human Pellagra, editorial, J. A. M. A. **109:** 1203 (Oct. 9) 1937.

78. Modern Views on Pellagra, editorial, Brit. Med. J. **1:** 127 (Jan. 15) 1938.

79. Spies, T. D.; Cooper, Clark, and Blankenhorn, M. A.: The Use of Nicotinic Acid in the Treatment of Pellagra. Read before the Central Society for Clinical Research, Chicago, Nov. 5, 1937.

80. Fouts, P. J.; Helmer, O. M.; Lepkovsky, Samuel, and Jukes, T. H.: Treatment of Human Pellagra with Nicotinic Acid, Proc. Soc. Exper. Biol. & Med. **37:** 405, 1937.

81. Harris, L. J.: Nicotinic Acid and the Pellagra-Preventing ("P. P.") Vitamin, Chem. & Ind. **56:** 1134 (Dec. 18) 1937 (from an address to the Birmingham University Biochemical Society, Dec. 9, 1937).

82. Spies, T. D.: The Response of Pellagrins to Nicotinic Acid, Lancet **1:** 252 (Jan. 29) 1938.

Smith, Ruffin and Smith[83] report recovery of one patient after the administration of 60 mg. of nicotinic acid daily for twelve days.

Spies, Cooper and Blankenhorn[84] treated seventeen cases (apparently including the fifteen cases previously reported by Spies[82]) with nicotinic acid. They also mentioned personal communications from other workers citing nine other favorable cases. These authors noted rapid improvement in the mucous membrane lesions and in erythematous skin lesions. They found that advanced, moist ulcerated skin lesions did not seem to be especially benefited and conclude that nicotinic acid is a potent therapeutic agent for treating the mucous membrane lesions of pellagra.

It appears, therefore, that nicotinic acid is either the pellagra-preventive vitamin, or a provitamin or that it is only one of two or more substances necessary for the prevention of all the symptoms described as pellagra or that it may be conjugated with other substances in the body into a more complex essential material. The latter possibility is suggested by the fact that Warburg's enzymes, diphosphonucleotide and triphosphonucleotide, each contain a molecule of nicotinic acid.

Smith, Ruffin and Smith[83] have suggested mixing nicotinic acid with table salt as a means of preventing pellagra. This suggestion appears to be premature because there is no information available on the effectiveness of nicotinic acid in the prevention of the disease and because so little is known about its physiologic action. It has been shown further that the diets on which pellagra develop are also deficient in other respects. Attempts to prevent the disease by the widespread use of nicotinic acid might allow the symptoms of vitamin B_1 deficiency, protein deficiency, riboflavin deficiency or mineral deficiency to replace pellagra as a nutritional problem in this country.

Sebrell, Onstott and Hunt[10] and Sebrell and Onstott[85] have found that dogs on a blacktongue-pro-

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83. Smith, D. T.; Ruffin, J. M., and Smith, S. G.: Pellagra Successfully Treated with Nicotinic Acid: A Case Report, J. A. M. A. **109**: 2054 (Dec. 18) 1937.

84. Spies, T. D.; Cooper, Clark, and Blankenhorn, M. A.: The Use of Nicotinic Acid in the Treatment of Pellagra, J. A. M. A. **110**: 622 (Feb. 26) 1938.

85. Sebrell, W. H., and Onstott, R. H.: Riboflavin Deficiency in Dogs, Pub. Health Rep. **53**: 83 (Jan. 21) 1938.

ducing diet to which substances containing the black-tongue-preventive factor are added will die unless riboflavin is also present.

Therefore, although it may be desirable at some future time to attempt to prevent pellagra by the use of nicotinic acid mixed with some commonly used southern article of diet, the safest procedure at present would appear to be to continue vigorously our efforts to prevent the disease by improving the diet, using nicotinic acid as individual circumstances indicate, and leaving the possibility of its uncontrolled administration to a large section of our population until additional information is available.

THE DIET IN THE TREATMENT OF PELLAGRA

In the present chaotic state of our knowledge about the B vitamins, the successful treatment of pellagra is founded on the use of adequate amounts of substances that have been shown experimentally to be most effective in the treatment and prevention of pellagra.

The earliest experiments of Goldberger and Tanner [18] demonstrated the value of the addition of milk, lean meat and legumes to the diet of the pellagrin. The data on the value of a variety of foods in pellagra are condensed in the table for ease of reference. Goldberger and Sebrell [86] emphasize the point that in mild cases of pellagra careful feeding is all that is needed. Spies [87] also finds that mild cases can be treated effectively by a well balanced diet of 4,000 calories or more daily, and adequate rest. The severer cases require more energetic treatment. The severer the case the more important it is that the patient shall eat, and since anorexia is a very common symptom it requires careful individual attention in order to make certain that the patient takes and retains the required food. The main reliance should be placed on large quantities of milk, lean meats, liver, and the other foods listed in the table as good sources of the pellagra-preventive vitamin.

The principal features, in brief, of the dietary management of pellagra recommended by Goldberger and Sebrell [86] in 1933 are as follows: A food intake of 3,000 calories a day should be the aim (in mild cases correction of the diet is all that is needed). Milk should be the principal item of diet; beef juice or meat soups and broths in small quantities at frequent intervals in gradually increasing amounts; solid food, particularly fresh lean meat and liver as soon as the patient's digestive system will permit; pure dried yeast in one-half to 1 ounce (7.5 to 15 Gm.) doses daily in milk, tomato juice or table syrup, and liver extracts in liberal doses in difficult cases. It should be particularly emphasized that ". . . success in treatment of the individual case will be in almost direct proportion to the attention devoted to the proper feeding of the patient." Spies, Chinn and McLester[88] used a similar scheme of treatment which differed essentially in an increase in the food intake to about 4,500 calories a day and an increase in the dose of yeast to from 180 to 270 Gm. daily. Forty-seven of fifty hospital patients with severe pellagra were successfully treated. It is probable that the three deaths were the result of complications.

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[86] Goldberger, Joseph, and Sebrell, W. H.: Pellagra, in Tice's Practice of Medicine **9**: 205. 1933.

[87] Spies, T. D.: The Medical Treatment of Early Pellagra, J. A. M. A. **105**: 1028 (Sept. 28) 1935.

In view of the foregoing, the essential points in the treatment of pellagra are:

1. Inclusion in the diet of at least 1 quart (liter) of milk daily.
2. A diet of from 3,000 to 4,000 calories or more daily containing, in addition to milk, lean meats, liver and other foods rich in the pellagra-preventive vitamin as given in the table.
3. Not less than 30 Gm. and up to 200 Gm. or more daily of pure dried powdered yeast, depending on the severity of the attack.
4. Liver extracts, either orally or parenterally, in very large daily doses in the severe cases.
5. Feedings at frequent intervals and careful, individual nursing, in order to see that the patient actually eats and retains the large amount of food necessary.
6. Symptomatic treatment as indicated.
7. Nicotinic acid orally or parenterally as indicated, in sufficient dosage to relieve acute symptoms rapidly.

PREVENTION OF RECURRENCE

Since the disease develops as a direct result of a deficient diet, it is to be expected that it will recur if the patient returns to his former diet, as soon as his supply

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88. Spies, T. D.; Chinn, Austin, and McLester, J. B.: Severe Endemic Pellagra: A Clinical Study of Fifty Cases with Special Emphasis on Therapy, J. A. M. A. **108**:853 (March 13) 1937. Spies, Chinn and McLester.[72]

of the preventive factor is again depleted. Recurrence, therefore, can be prevented only by bringing about a permanent change in the patient's dietary habits. The pellagrin should not be told to stop eating his cornbread, grits and syrup. These foods are cheap sources of energy, and it is useless and inadvisable to forbid the pellagrin to eat them. He should be advised to add to these foods the pellagra-preventive foods which he has some prospect of obtaining, such as green, leafy vegetables, fresh or canned milk or buttermilk, lean pork instead of fat pork, canned salmon, haddock and corned beef, poultry and rabbits. It is much more likely that he will follow this advice in some measure than that he will give up his established, basic dietary habits. Dietary advice should not be given in general terms. The dietary habits of each patient should be given careful, individual consideration in detail if a practical means of permanent improvement is to be devised. This can be accomplished best with the expert assistance of a nutritionist or well trained dietitian.

PREVENTION

The prevention of endemic pellagra is simple in theory but difficult in practice. If every normal person received enough of the foods containing the pellagra-preventive vitamin there would be no endemic pellagra. This means eating such foods as lean meat, milk, fish and a variety of vegetables containing the pellagra-preventive vitamin. Even if the patient is economically able to obtain these foods, such factors as availability and dietary habits and customs must be taken into consideration. Goldberger and Sydenstricker[89] and Wheeler and Sebrell[90] have pointed out the importance of the home production of foods containing the pellagra-preventive vitamin, especially during the late winter and early spring, and the use of dried, powdered yeast as the most important preventive measures in the areas of the United States in which pellagra is endemic. The widespread distribution of powdered yeast, either free or at the cheapest possible price, is the most effective method of immediate control known. However, this is only a palliative measure, which must be repeated each spring in order to keep down the incidence of the disease. DeKleine[91] also believes that home gardening and the distribution of pure, powdered yeast are two of the most important measures to be employed in the prevention of endemic pellagra. Permanent control can be obtained only by bringing about permanent changes in dietary habits.

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[89] Goldberger, Joseph, and Sydenstricker, Edgar: Pellagra in the Mississippi Flood Area: Report of An Inquiry Relating to the Prevalence of Pellagra in the Area Affected by the Overflow of the Mississippi and Its Tributaries in Tennessee, Arkansas, Mississippi and Louisiana in the Spring of 1927, Pub. Health Rep. **42**: 2706 (Nov. 4) 1927.

[90] Wheeler, G. A., and Sebrell, W. H.: The Control of Pellagra, J. A. M. A. **99**: 95 (July 9) 1932.

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[91] DeKleine, William: Recent Trends in Pellagra, Am. J. Pub. Health. **27**: 595 (June) 1937.

CHAPTER XVII

THE CHEMISTRY OF VITAMIN C

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IDENTIFICATION

A primary requisite for studying the chemical nature of vitamin C was provided by Holst and Frölich[1] when they observed that the guinea pig could be used as an experimental animal for the study of scurvy. The experimental diets and general technic developed by Cohen and Mendel[2] and La Mer, Campbell and Sherman[3] then made it possible to measure antiscorbutic activity in a satisfactory quantitative manner, free from interference by deficiencies in other essential nutrients. Attempts to isolate the vitamin from natural products encountered great difficulty, however, because of its extreme sensitiveness to destruction by oxidation.

By 1931 many investigators, particularly Zilva and his associates,[4] Bezssonoff and his associates[5] and King and his associates[6] had succeeded in concentrating the vitamin to such a degree that approximately 1 to 2 mg. of solids daily served to protect young guinea pigs from scurvy. The isolation of the vitamin from lemons and its identification as "hexuronic acid," having a protective level of 0.5 mg. a day, by Waugh and King[7]

From the Department of Chemistry, University of Pittsburgh.

[1] Holst, A., and Frölich, T.: Experimental Studies Relating to Ship Beriberi and Scurvy, J. Hyg. **7:** 634, 1907.

[2] Cohen, B., and Mendel, L. B.: Experimental Scurvy of the Guinea Pig in Relation to the Diet, J. Biol. Chem. **35:** 425 (July) 1918.

[3] La Mer, V. K.; Campbell, H. L., and Sherman, H. C.: Quantitative Determination of the Antiscorbutic Vitamin, J. Am. Chem. Soc. **44:** 165 (Jan.) 1922.

[4] Zilva, S. S.: The Antiscorbutic Fraction of Lemon Juice: VIII, Biochem. J. **23:** 1199 (Nov.) 1929.

[5] Bezssonoff, N.: Vitamin Color Tests, Bull. Soc. chim. biol. **11:** 294 (Feb.) 1929.

[6] Smith, F. L., and King, C. G.: The Preparation and Storage of Vitamin C Concentrates from Lemon Juice, J. Biol. Chem. **94:** 491 (Dec.) 1931. Svirbely, J. L., and King, C. G.: The Preparation of Vitamin C Concentrates from Lemon Juice, ibid. **94:** 483 (Dec.) 1931.

[7] Waugh, W. A., and King, C. G.: The Chemical Nature of Vitamin C, Science **75:** 357 (April 1) 1932; The Isolation and Identification of Vitamin C, J. Biol. Chem. **97:** 325 (July) 1932; Proc. Am. Soc. Biol. Chem., April 1932.

in 1932 was followed within a few weeks by a report from Svirbely and Szent-Gyorgyi [8] that 1 mg. daily of "hexuronic acid" prepared from adrenal glands was protective against scurvy. The protective value of hexuronic acid prepared from adrenal glands by Kendall,[9] using an entirely different procedure, was then shown by Waugh and King [10] to be identical with that of the product from lemons, thereby essentially removing any question of contamination of the original crystals by another active substance. In their next paper Svirbely and Szent-Gyorgyi [11] also found the minimum protective level of their product to be 0.5 mg. a day. Within a short time the identity of the vitamin was independently confirmed by several laboratories, particularly

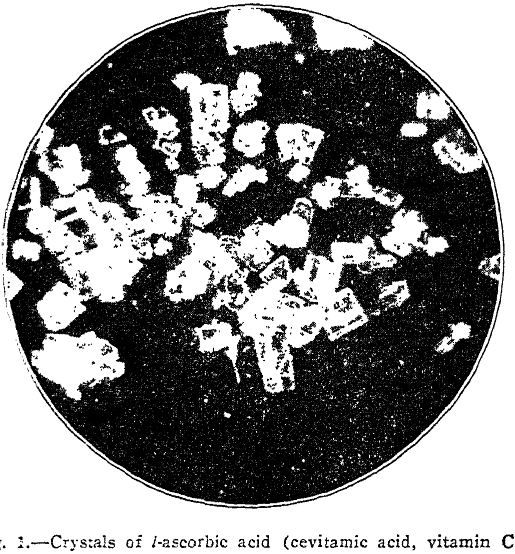

Fig. 1.—Crystals of *l*-ascorbic acid (cevitamic acid, vitamin C).

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[8] Svirbely, J. L., and Szent-Gyorgyi, Albert: Hexuronic Acid as the Antiscorbutic Factor, Nature **129**: 576 (April 16) 1932; Biochem. J. **26**: 865 (No. 3) 1932.

[9] Kendall, E. C.: The Consideration of Some of the Glands of Internal Secretion from a Chemical Viewpoint, Endocrinology **15**: 357 (Sept.-Oct.) 1931.

[10] Waugh, W. A., and King, C. G.: The Vitamin C Activity of Hexuronic Acid from Suprarenal Glands, Science **76**: 630 (Dec. 30) 1932.

[11] Svirbely, J. L., and Szent-Gyorgyi, Albert: The Chemical Nature of Vitamin C, Biochem. J. **27**: 279 (Feb.) 1933.

those of Tillmans,[12] Vedder,[13] Nelson,[14] Harris [15] and von Vargha.[16] The compound $C_6H_8O_6$, having the same general formula and many properties in common with other hexuronic acid lactones, had been prepared from cabbage, oranges and adrenal glands in 1928 by Szent-Gyorgyi [17] in studying tissue respiration systems, but there was no knowledge of its antiscorbutic value previous to the work published in 1932.

It may be interesting to note that isolation of vitamin C resulted, in one laboratory, from four years of systematic fractionation of lemon juice, based entirely on biologic assays, and that in another laboratory biologic assay for vitamin function was reported five years subsequent to the isolation of the vitamin as hexuronic acid, a silver nitrate-reducing agent in tissues, and two years after its negative assay as a hormone.

STRUCTURE AND SYNTHESIS

The structural formula of the vitamin was established in 1933, primarily by Haworth, Hirst and his associates,[18] Karrer and his associates,[19] Micheel and Kraft [20] and von Euler and Klussmann,[21] using material obtained from adrenal glands and paprika, supplied largely from Szent-Gyorgyi's laboratory. Before the structure was established with certainty, Reichstein, Grussner and Oppenauer [22] succeeded in synthesizing the *d*- and later the *l*- form of the acid. Some delay and confusion occurred in working out a satisfactory graphic formula, because the vitamin represented an entirely new type of configuration for a hexuronic acid lactone. Before the structural formula had been established, so that the acid could be named according to its relationship to known sugars, Haworth and Szent-Gyorgyi suggested the name "ascorbic acid"[23] to connote its antiscorbutic nature. The American Medical Association introduced the name "cevitamic acid" expressly to avoid this connotation.

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[12] Tillmans, J.; Hirsch, P., and Vaubel, R.: The Reducing Value of Plant Foods and Its Indication of Vitamin C, Ztschr. Untersuch. Lebensm. **65:** 145 (Feb.) 1933; Biochem. Ztschr. **250:** 312, 1932.

[13] Vedder, E. B.: A Study of the Antiscorbutic Vitamin, Mil. Surgeon **71:** 505 (Dec.) 1932.

[14] Nelson, E. K.: The Isolation of Hexuronic Acid, Science **76:** 345 (Oct. 14) 1932.

[15] Harris, L. J., and Ray, S. H.: Specificity of Hexuronic Acid as Antiscorbutic Factor, Biochem. J. **26:** 580 (April) 1933.

[16] von Vargha, L.: Monoacetone Hexuronic Acid, Nature **130:** 847 (Dec. 4) 1932; Triphenylmethyl Derivative of Vitamin C, Nature **131:** 363 (March 11) 1933.

[17] Szent-Gyorgyi, Albert: Observations on the Functions of the Peroxidase Systems and the Chemistry of the Adrenal Cortex, Biochem. J. **22:** 1387 (Dec.) 1928.

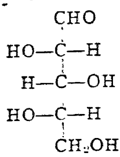
[18] Herbert, R. W.; Percival, E. G. V.; Reynolds, R. J. W.; Smith, F., and Hirst, E. L.: Constitution of Ascorbic Acid, J. Soc. Chem. Ind. **52:** 221, 481 (Nos. 1, 2) 1933. Cox, E. G., and Goodwin, T. H.: Crystalline Structure of the Sugars: III. Ascorbic Acid and Related Compounds, J. Chem. Soc. **1936:** 769.

[19] Karrer, Paul; Schoof, K., and Zehender, F.: Concerning Vitamin C, Helv. chim. acta **16:** 1161 (9) 1933.

[20] Micheel, Fritz, and Kraft, Kurt: The Constitution of Vitamin C, Ztschr. physiol. Chem. **222:** 235 (No. 1) 1933.

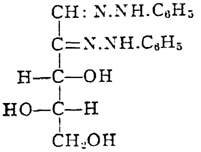
[21] Karrer, Paul; von Euler, H., and Hellstrom, H.: Vitamin C, Arkiv. kemi. mineral. Geol. **11B,** 6 pp. (6) 1933. von Euler, H., and Klussmann, H.: Biochemical Experiments with Vitamin C and Sugar Derivatives, ibid., 6 pp. (7) 1933.

[22] Reichstein, T.; Grussner, A., and Oppenauer, R.: Synthesis of *d*- and *l*-Ascorbic Acid, Helv. chim. Acta **16:** 1019 (No. 8) 1933.



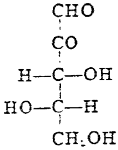
$$\begin{array}{c} CHO \\ HO-C-H \\ H-C-OH \\ HO-C-H \\ CH_2OH \end{array}$$

I. l-xylose



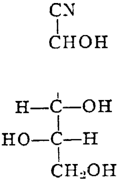
$$\begin{array}{l} CH{:}N.NH.C_6H_5 \\ C{=}N.NH.C_6H_5 \\ H-C-OH \\ HO-C-H \\ CH_2OH \end{array}$$

II. l-xylosazone



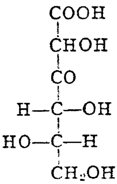
$$\begin{array}{c} CHO \\ CO \\ H-C-OH \\ HO-C-H \\ CH_2OH \end{array}$$

III. l-xylosone



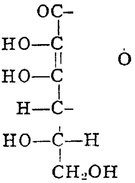
$$\begin{array}{c} CN \\ CHOH \\ \\ H-C-OH \\ HO-C-H \\ CH_2OH \end{array}$$

IV. nitrile of l-xylosone



$$\begin{array}{c} COOH \\ CHOH \\ CO \\ H-C-OH \\ HO-C-H \\ CH_2OH \end{array}$$

V. 3-ketogulonic acid (intermediate, not isolated)



$$\begin{array}{cc} OC- & \\ HO-C & \\ \| & O \\ HO-C & \\ H-C- & \\ HO-C-H & \\ CH_2OH & \end{array}$$

VI. vitamin C (ascorbic or cevitamic acid)

Synthesis was first accomplished from *l*-xylose, I. On treatment with phenyl hydrazine *l*-xylose yields the osazone, II. This compound on hydrolysis yields *l*-xylosone, III. Treatment of the latter with hydrocyanic acid produces the nitrile of the *l*-xylosone, IV, which on hydrolysis is converted first into the intermediate 2-ketogulonic acid, V. In an acid medium lactonization and enolization occur and vitamin C itself, VI, is produced.

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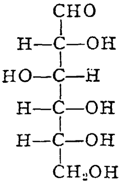
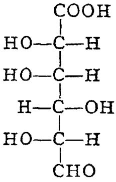
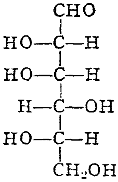
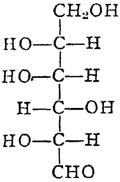
[23] Szent-Gyorgyi, Albert, and Haworth, W. N.: "Hexuronic Acid" (Ascorbic Acid) as the Antiscorbutic Factor, Nature **131**: 23 (Jan. 7) 1933.

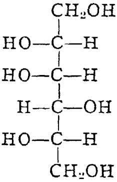
Several different methods of synthesis have been published in detail and others have been disclosed only in patents. The catalytic and electrolytic reduction of *d*-glucose to sorbitol on a commercial scale, followed by selective fermentation to *l*-sorbose, provides a basis for commercial synthesis at low cost. A number of procedures have been followed for converting sorbose to the intermediate sorburonic or 2-ketogulonic acid. The more important structurally related compounds are *d*-glucose, *d*-glucuronic acid, *l*-gulose, sorbitol, *l*-sorbose and sorburonic acid.

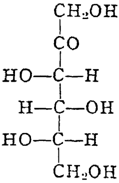

d-glucose

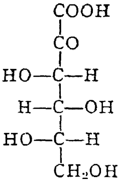

d-glucuronic acid


l-gulose


d-glucose


sorbitol


l-sorbose


2-ketogulonic acid
or
sorburonic acid

Comparison of the formulas for *d*-glucose, *l*-gulose and *l*-sorbose serves to illustrate the transition in series from *d*- to *l*- in going through the *d*-glucose → sorbitol → sorbose steps, since the *d*- or *l*- nomenclature is based on the configuration of the asymmetric carbon farthest from the saccharide group. Antiscorbutic activity is contingent on the *d*- configuration of C-4 (responsible for its dextrorotation) and the *l*- configuration of C-5. A number of sugar derivatives having structures analogous to that of ascorbic acid (2,3-enediol-4-lactone) have been prepared and shown to have similar chemical properties. Zilva [24] has given a good summary of the relative physiologic values of closely related synthetic substances, the most active being *l*-rhamnoascorbic acid, which had one fifth of the antiscorbutic value of ascorbic acid. None of the other compounds examined had more than one tenth of the value of ascorbic acid. *d*-Ascorbic acid had no antiscorbutic value.

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[24] Zilva, S. S.: The Behavior of *l*-Ascorbic Acid and Chemically Related Compounds in the Animal Body, Biochem. J. **29**: 1612 (Sept.) 1935.

TYPE REACTIONS

The acidic properties of the vitamin are due to dissociation of an enolic hydrogen rather than to opening of the lactone ring. Methylation with diazomethane involves the enolic groups (C-2 and C-3) first, and further treatment with dimethylsulfate forms ethers from the other two OH groups (C-5 and C-6). Esterification of the alcohol groups on C-5 and C-6 can be accomplished readily with acyl halides. The carboxyl group does not enter into any of the common laboratory reactions of the vitamin in either aqueous or alcoholic solution. The lactone ring of dehydroascorbic acid apparently opens more readily than that of ascorbic acid. Condensation with aldehydes, acetone and other ketones takes place under the influences of very mild dehydrating agents, giving rise to stable compounds that can be crystallized readily from acetone, alcohol and other organic solvents. Triphenylmethylchloride forms an ether (trityl) readily on carbon-6. Heating with strong mineral acids gives rise to furfural and carbon dioxide.

The reactions of greatest general interest are those dealing with oxidation and reduction. Barron, De Meio and Klemperer have shown that in aqueous solution below p_H 7.6 the vitamin is not oxidized on exposure to air unless the reaction is catalyzed by traces of copper[25] or some other activating agency. Although copper had been widely recognized as a very effective catalyst, their results were of special interest in showing that the vitamin is nonautoxidizable within the normal p_H range of plant and animal tissues. The interdependence of p_H, anions and concentration of copper in their relation to the rate of aerobic oxidation has been demonstrated by Lyman, Schultze and King.[26] As would be expected from its oxidation-reduction potential, the vitamin is reversibly oxidized by a great many organic compounds such as methylene blue, quinones and the indophenol dyes, and by inorganic reagents such as Cu^{++}, Fe^{+++}, Hg^{++}, Ag^{+}, Mn^{+++}, $Fe(CN)_6^{---}$, NO_3^{-}, WO_4^{--}, H_2O_2, and I_2. A discussion of the use of oxidizing agents for the quantitative estimation of vitamin C in tissues will be found in the section on methods of assay. The dry crystals of vitamin are stable on exposure to air and daylight at room temperature through a period of several years. They tend to become buff colored without undergoing appreciable decomposition. In aqueous solution, however, the rate of aerobic oxidation is greatly accelerated by exposure to light, especially in the presence of flavins.

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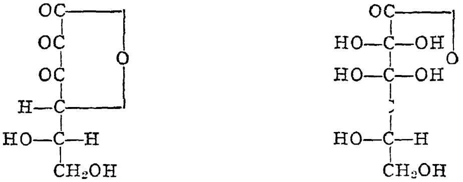
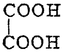
[25] Barron, E. S. G.; De Meio, R. H., and Klemperer, F.: Biological Oxidations: V. Copper and Hemochromogens as Catalysts for the Oxidation of Ascorbic Acid, J. Biol. Chem. **112**: 625 (Jan.) 1935-1936.



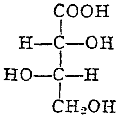
VII. dehydroascorbic acid

(ketone formula) (hydrate formula)



$$\begin{array}{c}COOH\\ |\\ COOH\end{array}$$

VIII. oxalic acid



$$\begin{array}{c}COOH\\ |\\ H—C—OH\\ |\\ HO—C—H\\ |\\ CH_2OH\end{array}$$

IX. l-threonic acid

Dehydroascorbic acid, VII, the reversibly oxidized product formed from ascorbic acid, is fairly stable in acidic aqueous solutions, e. g. below p_H 4, but in faintly acid (p_H above 4), neutral or alkaline solutions it undergoes an irreversible rearrangement, giving rise to another strong reducing substance. It is this second strong reducing substance, as shown particularly by the work of Borsook and his associates,[27] that gives rise to oxalic acid, VIII, and *l*-threonic acid, IX, on further oxidation.

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[26] Lyman, C. M.; Schultze, M. O., and King, C. G.: The Effect of Metaphosphoric Acid and Some Other Inorganic Acids on the Catalytic Oxidation of Ascorbic Acid, J. Biol. Chem. **118**: 757 (May) 1937.

[27] Borsook, Henry; Davenport, H. W.; Jeffreys, C. E. P., and Warner, R. C.: The Oxidation of Ascorbic Acid and Its Reduction in Vitro and in Vivo, J. Biol. Chem. **117**: 237 (Jan.) 1937.

Reduction of dehydroascorbic acid can be accomplished readily by H_2S, cysteine, glutathione and the "fixed-SH" groups associated with proteins. The rates of reduction are markedly dependent on the relative concentrations and the p_H of the solutions, however. Metals reacting with acids, or hydrogen under pressure with common catalysts do not reduce the product with good yields.

PHYSICOCHEMICAL PROPERTIES

When adequately protected from oxidation, the vitamin can be crystallized from solution in water or the lower alcohols, acetone, ethyl acetate and many other organic solvents. The colorless crystals tend to form dense radiating clusters but may form characteristic plates or needles (monoclinic, pseudo-orthorhombic), depending on the solvent and the rate of crystallization.

Its physical constants are: melting point 192 C.; specific rotation +24 degrees in water, 48 degrees in alcohol, without mutarotation; acidic dissociation $pk_1 = 4.17$ (Harris et al.) (or 4.20 cf. Ball), $pk_2 = 11.57$; absorption maximum 260 millimicrons in water, 263 millimicrons in alcohol (Karrer, Hirst, et al.); oxidation-reduction potential (Borsook) at p_H 4.0 and 35 degrees. $E\frac{1}{0} = +0.166$ V. Ball gives +0.390 volts for the normal potential of ascorbic acid at 30 degrees, and five minutes for the half life of dehydroascorbic acid at p_H 7.24, 30 degrees. For a detailed discussion of the oxidation-reduction potentials under various conditions, the recent papers of Borsook and his associates[27] and of Ball[28] should be consulted.

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[28] Ball, E. G.: Studies on the Oxidation-Reduction of Ascorbic Acid, J. Biol. Chem. **118**: 219 (March) 1937.

CHAPTER XVIII

THE PHYSIOLOGY OF VITAMIN C

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ORIGIN AND PHYSIOLOGIC CONTROL

Dry seeds contain practically no ascorbic or dehydroascorbic acid but, when they are moistened and warmed, ascorbic acid appears within a few hours in the areas where the sprouting processes are apparently initiated.[1] Unripe, rapidly growing seeds, such as green peas, are relatively rich sources of the vitamin, but as ripening advances the concentration approaches zero. An extensive literature [2] indicates that all actively growing parts of the higher plants (roots, stems, buds and pods), all fresh green leaves, many of the algae [3] and perhaps even bacteria [4] contain significant quantities of the vitamin. The carotenoid pigments are frequently accompanied by high concentrations of ascorbic acid in both plant and animal tissues, as in rose hips, paprikas and corpora lutea, but there are many exceptions to such a relationship, as in vitamin rich, nonpigmented sprouts.

Of the extensive number of animals studied, only man, the other primates and guinea pigs do not have a capacity to synthesize ascorbic acid. There is no convincing evidence that infants, placental tissue or any of the tissues of the animals named can synthesize ascorbic acid at any stage in their development. The evidence that is frequently cited in the literature to prove such a synthesis can be interpreted as well, and in most cases better, on the basis of selective conservation of the vitamin in the more vital tissues, such as

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From the University of Pittsburgh.

1. Glick, David: Quantitative Distribution of Ascorbic Acid in the Growing Barley Embryo, Ztschr. f. physiol. Chem. **245**: 211 (Feb.) 1937. von Hausen, Synnöve: Die Rolle des Vitamins C beim Wachstum der höheren Pflanzen, Biochem. Ztschr. **288**: 378 (Dec.) 1936.

2. King, C. G.: Vitamin C: Ascorbic Acid, Physiol. Rev. **16**: 238 (April) 1936.

3. Norris, E. R.; Simeon, M. K., and Williams, H. B.: The Vitamin B and C Content of Marine Algae, J. Nutrition **13**: 425 (April) 1937.

4. Bourne, G., and Allen, R.: Vitamin C in Lower Organisms, Australian J. Exper. Biol. & M. Sc. **13**: 165 (Feb.) 1935.

the pituitary body, during a period of general body depletion. Furthermore, it is known that both human beings and guinea pigs are subject to scurvy during gestation and lactation. The early synthesis of vitamin C in chick embryos [5] demonstrates a tissue reaction that is retained by the adult organism.

The claim that mannose could serve as a precursor of vitamin C in rat tissue could not be confirmed.[6] There is still no clear evidence concerning the nature of the substance from which vitamin C is synthesized in animals or plants, nor is there any indication of the specific agencies or tissues responsible for the synthesis. The earlier known metabolites in plants and animals that are most closely related to the vitamin are glucuronic and galacturonic acids. Either of these could give rise to the vitamin by replacement of the carbonyl group from the first to the fourth or fifth carbon, followed by lactonization and enolization. Recent investigations in the author's laboratory have shown, however, that volatile constituents of plant and animal "unsaponifiable matter" (lipid in nature) can serve as the precursor of ascorbic acid in the body of the rat.

The distribution of vitamin C in the human body [7] is comparable with that in the guinea pig and in animals that synthesize a continuous supply in their own tissues, such as the rat and the chicken.[8] Giroud [9] has studied an extensive number of invertebrates. In general, the tissues that are characterized by a high metabolic activity have a high vitamin C content. Thus the individual tissues of young animals are consistently richer in vitamin C than those of older animals, and in special cases in which one can follow the changing vitamin content during cycles of activity and atrophy the relationship to functional activity is striking. An excellent illustration of this principle is provided by the microtitration studies of Glick and Biskind on thymus [10] and corpus luteum.[11]

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[5] Ray, S. N.: A Note on the Presence of Vitamin C in the Chick Embryo, Biochem. J. **28:** 189, 1934.

[6] Hawthorne, J. R., and Harrison, D. C.: Mannose as a Possible Precursor of Ascorbic Acid in the Tissues of the Rat, Biochem. J. **31:** 1061 (July) 1937. Scheunert, A., and Schieblich, M.: Vitamin C Production in the Rat by Various Nutrients, Ztschr. f. physiol. Chem. **246:** 272 (April) 1937.

[7] Yavorsky, Martin; Almaden, Philip, and King, C. G.: The Vitamin C Content of Human Tissues, J. Biol. Chem. **106:** 525 (Sept.) 1934.

[8] Bessey, O. A., and King, C. G.: The Distribution of Vitamin C in Plant and Animal Tissues, and Its Determination, J. Biol. Chem. **103:** 687 (Feb.) 1933. Daniel, Esther P., and Munsell, Hazel E.: Vitamin Content of Foods, Miscellaneous Circular 275, U. S. Department of Agriculture, June 1937.

[9] Giroud, Antoine, and Rakoto-Ratsimamanga, A.: Distribution of Vitamin C in Invertebrates, Bull. Soc. chim. Biol. **18:** 375 (Dec.) 1936.

Normal tissues from different animals do not fall in exactly the same sequence with regard to concentration, but the following order corresponds approximately to a general decreasing concentration of vitamin C: pituitary body (2.6 mg. per gram in the pars intermedia), corpus luteum, adrenal cortex, young thymus, liver, brain, testes, ovaries, spleen, thyroid, pancreas, salivary glands, lung, kidney, intestinal wall, heart, muscle, spinal fluid and blood. Normally the average content of human blood plasma is about 1.2 mg. of vitamin per hundred cubic centimeters,[12] but depletion reduces this to about 0.8 mg. as the "prescorbutic state" is reached and to approximately 0.5 mg. with the appearance of "clinical scurvy." Tumor tissue varies greatly with different types but generally shows a high content during rapid growth and a characteristic decrease with necrosis.[13]

In relation to infant nutrition and from the point of view of comparative physiology, it is both interesting and important to note that human milk normally contains from four to five times more vitamin C than cow's milk. The human infant has a high dietary requirement to meet the need for rapid growth and general metabolism, but the calf is not dependent on a dietary supply, its current requirement being met by tissue synthesis, as in the rat and the chick. When the human mother's diet is seriously deficient in antiscorbutic foods, however, the concentration in the milk gradually falls to approximately that of cow's milk,[14] and when the vitamin C

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10. Glick, David, and Biskind, G. R.: The Concentration of Vitamin C in the Thymus in Relation to Its Histologic Changes at Different Stages of Development and Regression, J. Biol. Chem. **114:** 1 (May) 1936.

11. Biskind, G. R., and Glick, David: The Vitamin C Concentration of the Corpus Luteum with Reference to the Stage of the Estrus Cycle and Pregnancy, J. Biol. Chem. **113:** 27 (Feb.) 1936.

12. Abt, A. B.; Farmer, C. J., and Epstein, I. M.: Normal Cevitamic (Ascorbic) Acid Determinations in Blood Plasma and Their Relationship to Capillary Resistance, J. Pediat. **8:** 1 (Jan.) 1936. Stephens, D. J., and Hawley, Estelle E.: The Partition of Ascorbic Acid in Blood, J. Biol. Chem. **115:** 653 (Oct.) 1936.

13. Musulin, R. R.; Silverblatt, Ethel; King, C. G., and Woodward,

477 (March) 1937. Kellie, A. E., and Zilva, S. S.: The Indophenol-Reducing Substance in Jensen Rat Sarcoma, Biochem. J. **30:** 1216 (July) 1936.

14. Selleg, Iva, and King, C. G.: The Vitamin C Content of Human Milk and Its Variation with Diet, J. Nutrition **11:** 599 (June) 1936. Neuweiler, W.: Vitamin C Content of Milk, Ztschr. f. Vitaminforsch. **4:** 39 (Jan.) 1935; the Vitamin C Requirement During Pregnancy and Lactation, Klin. Wchnschr. **14:** 1793 (Dec. 14) 1935.

intake is increased, the antiscorbutic value of the milk returns to normal.

METABOLISM

Harris and Ray[15] and later many other writers have shown that there is a continuous but decreasing urinary excretion of vitamin C when there is no dietary intake. When the body has become depleted of its normal vitamin C content, readministration of the vitamin first causes a restorage in the various tissues, and when the deficiency has been made up, urinary excretion of the excess again follows. Such balance experiments provide a reasonably satisfactory means of clinically evaluating the various stages of malnutrition. The net loss, or difference between intake and excretion, for a person in a state of "saturation" may be taken as an approximation of the quantity normally destroyed, or "burned," in the body daily. This value apparently varies considerably with different persons but may be estimated to fall in the range of from 25 to 50 mg. daily. It appears from the work of Hawley and her associates[16] that the quantity of vitamin excreted may be varied by merely changing the acid-base balance of the food intake.

GENERAL PHYSIOLOGIC RELATIONSHIPS

The most clearly established functional role of vitamin C in animal tissues is its relationship to the physical state of the "intercellular material," as described by Wolbach and his associates. A detailed discussion of this field of study will be found in the section on pathology. The relationship of vitamin C to calcium metabolism is intimately associated with this phenomenon and is discussed in the same section. Both roles are clearly of major importance in relation to growth and repair of bones and teeth, and it is evident that other tissues, such as cartilage and white fibrous tissue, are markedly affected in a similar manner. The chemical mechanism through which the vitamin brings about this control of metabolism is still unknown.

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[15] Harris, L. J., and Ray, S. N.: Diagnosis of Vitamin C Subnutrition by Urine Analysis, with a Note on the Antiscorbutic Value of Human Milk, Lancet **1:** 71 (Jan. 12) 1935. von Euler, Hans, and Malmberg, Maj: Excretion and Accumulation of Vitamin C in the Human and Animal Organism, Biochem. Ztschr. **279:** 338, 1935.

[16] Hawley, Estelle E.; Frazer, J. P.; Button, L. L., and Stevens, D. J.: The Effect of the Administration of Sodium Bicarbonate and of Ammonium Chloride on the Amount of Ascorbic Acid Found in the Urine, J. Nutrition **12:** 215 (Aug.) 1936.

From the close relationship between growth and vitamin C content it is reasonable to conclude that the vitamin has an essential role in the over-all growth processes of animals and plants. Work by Bonner and Axtman indicates that ascorbic acid serves as a powerful growth stimulant for young plant embryos that have been isolated from their seed reserve food supply.

Using the acid and silver nitrate staining technic, Bourne and Allen[4] and Giroud and Leblond[17] made detailed studies of the distribution of ascorbic acid within the cell, as well as the relative distribution in whole tissues. In an extensive series of papers from the two laboratories they have emphasized the association of the vitamin with the Golgi apparatus and mitochondria. Their results have served to emphasize the selective retention of the vitamin in certain parts of the cell and in vital tissues such as the pituitary body.

The anemia that accompanies depletion of vitamin C is thought to be due primarily to dysfunction of the tissues that form the red blood cells rather than to excessive destruction of cells or specific failure in synthesis of hemoglobin. The decrease in hemoglobin is roughly parallel to the decrease in red blood cells and is accompanied by a moderate decrease in clotting time and in the number of blood platelets.

When guinea pigs are given diets free from vitamin C or low intakes, impairment of physiologic functions can be demonstrated before growth is severely restricted or external evidences of scurvy appear. This "prescorbutic state" is clearly of common occurrence in the general population and in relation to human physiology and public health apparently represents an important field of investigation. Animals in such a border stage of deficiency are more sensitive to injury from injections of diphtheria toxin,[18] and their normal metabolic activity, as measured by dextrose tolerance, is moderately lowered.[19] Many, if not all, of the reports indicating detoxification by ascorbic acid in vitro apparently were due to underestimating the importance of p_H effects.[19] The arteries, teeth and adrenals are particularly sensitive to injury by toxins when the tissues are depleted of their normal vitamin C reserves. Resistance against infections is also lowered in the prescorbutic condition,[20] and it is worthy of note that bacterial toxins can cause losses of from 50 to 85 per cent of the vitamin normally contained in the adrenals.[21]

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[17] Giroud, Antoine, and Leblond, C. P.: Histologic Study of Renal Elimination of Ascorbic Acid, Anat. Rec. **68:** 113 (April 25) 1937.

Toxin, Proc. Soc. Exper. Biol. & Med. **32:** 1157 (April) 1935. Sigal and King.[19]

[19] Sigal, Alex, and King, C. G.: The Influence of Vitamin C Deficiency upon the Resistance of Guinea Pigs to Diphtheria Toxin, J. Pharmacol. & Exper. Therap. **61:** 1 (Sept.) 1937; Vitamin C and Diphtheria Toxin, ibid. **59:** 468 (April) 1937.

The claim by Szent-Györgyi that a new factor called "vitamin P" is responsible for the antihemorrhagic effect attributed to vitamin C has been shown to be erroneous. The postulated "vitamin P" apparently does not exist.[22]

SPECIFIC RELATIONSHIPS TO ENZYMES

At the present time it is impossible to indicate with certainty any specific relationships between vitamin C and the enzymes in animal tissues so far as normal physiologic processes are concerned; but many papers record activating and inhibiting effects on enzymes in vitro and it is not unlikely that some of the observed effects [2] will prove to be physiologically significant.

Both ascorbic acid and dehydroascorbic acid, and a number of their cation complexes (e. g., with Fe^{++}, Cu^{++} and Mn^{++}) have been reported to exert marked effects on specific enzymes, and studies on cathepsin, papain,[23] amylase,[24] arginase, catalase, urease,[25] tyrosinase and nuclease [26] have offered special promise. It is likely that many (but probably not all) of the observed effects are more or less fortuitous observations in vitro, with little bearing on the role of the vitamin in vivo. The results from different laboratories have not been in good agreement, and it is evident in some cases that the nonspecific acidity and reducing effects of the vitamin have caused changes that were attributed specifically to the vitamin.

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[20] Perla, David, and Marmorston, Jessie: Role of Vitamin C in Resistance, Arch. Path. **23:** 683 (May) 1937.

[21] Lyman, C. M., and King, C. G.: The Effect of Diphtheria Toxin on the Vitamin C Content of Guinea Pig Tissues, J. Pharmacol. & Exper. Therap. **56:** 209 (Feb.) 1936. Harris, L. J.; Passmore, R., and Pagel, W.: Vitamin C and Infection, Lancet **2:** 183 (July 24) 1937. Torrance, C. C.: A Comparison of the Effect of Different Toxic Bacterial Products upon the Adrenals of Guinea Pigs, J. Bact. **33:** 645 (June) 1937.

[22] Zilva, S. S.: Vitamin P, Biochem. J. **31:** 915 (June) 1937.

[23] Maschmann, Ernst: Observations on the Activation of Plant Proteases, Ztschr. f. physiol. Chem. **228:** 141, 1934.

[24] Purr, Arnulf: The Influence of Vitamin C on Plant and Animal Amylases, Biochem. J. **28:** 1141 (June) 1934.

[25] Leuthardt, F., and Koller, F.: Concerning the Activation of

125: 283, 1937.

[26] Mayer, Mary E., and Voegtlin, Carl: The Chemistry of Cell Division, Am. J. Cancer **25:** 780 (Dec.) 1935.

The catalyst frequently called "ascorbic acid oxidase," which occurs widely in plant tissues, has been shown to have the composition and properties of a protein combined with copper, in which the copper serves as the active catalyst.[27] Since there is evidence that the catalyst, or enzyme, is not specific for vitamin C as a substrate,[27a] the name should be used in a restricted sense only.

The cytochrome-indophenol oxidase system has been shown to act as a catalyst for the aerobic oxidation of ascorbic acid, and there is evidence that this system is chiefly responsible for the observed (slow) aerobic oxidation of the vitamin in excised animal tissues.[28] In view of Barron's [29] work, the hemochromogens are also of interest.

Although the theory is widely held that vitamin C serves primarily as a hydrogen-transport agent, or respiratory catalyst, there is no clear-cut evidence to support the theory in relation to animal tissues.[30] The experiments of Hopkins and Morgan [31] and of Borsook and his co-workers [32] strongly support the theory in relation to plants, however, and place special emphasis on the role of glutathione in such a cycle in animal tissues.[28] A strong point against the theory that vitamin C functions as a major respiratory catalyst is the fact that depleted tissues do not show a decreased respiration capacity, and when ascorbate is added to the depleted tissues there is no rise in the true oxygen consump-

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27. Stotz, Elmer; Harrer, C. J., and King, C. G.: A Study of Ascorbic Acid Oxidase in Relation to Copper, J. Biol. Chem. **119**: 511 (July) 1937.

27a. Johnson, S. W., and Zilva, S. S.: The Relation Between the Rate of Enzymic Oxidation and the Stereochemical Structure of Ascorbic Acid and Its Analogues, Biochem. J. **31**: 1366 (No. 6) 1937.

28. Stotz, Elmer; Schultze, M. O.; Harrer, C. J., and King, C. G.: The Oxidation of Ascorbic Acid in the Presence of Guinea Pig Liver, J. Biol. Chem. **122**: 407 (Jan.) 1938.

29. Barron, E. S. G.; De Meio, R. H., and Klemperer, F.: Biological Oxidations: V. Copper and Hemochromogens as Catalysts for the Oxidation of Ascorbic Acid, J. Biol. Chem. **112**: 625 (Jan.) 1936.

30. Stotz, Elmer; Harrer, C. J.; Schultze, M. O., and King, C. G.: Tissue Respiration Studies on Normal and Scorbutic Guinea Pig Liver and Kidney, J. Biol. Chem. **120**: 129 (Aug.) 1937.

31. Hopkins, F. G., and Morgan, E. J.: Some Relations Between Ascorbic Acid and Glutathione, Biochem. J. **30**: 1446 (Aug.) 1936.

32. Borsook, Henry Davenport, H. W.; Jeffreys, C. E. P., and Warner, R. C.: The Oxidation of Ascorbic Acid and Its Reduction in Vitro and in Vivo, J. Biol. Chem. **117**: 237 (Jan.) 1937.

tion. The livers of scorbutic animals show an increased oxygen consumption which is comparable to that shown by the entire body.[30]

Recently Green and Richter[33] reported that ascorbic acid acts as an inhibitor in the adrenalin-adrenochrome oxidation system in heart tissue. Lemberg, Cortis-Jones and Norrie[34] have studied a coupled type of oxidation of ascorbic acid and hemochromogens, extending the earlier work of Barron[29] and Karrer, especially correlating the coupled oxidation with verdohemochromogen formation from hematin.

Great interest is attached to the finding by Ecker and associates of a close relationship between vitamin C and guinea pig blood complement.[35] The reversible oxidation-reduction behavior of complement, as a single protein substance, was found to be dependent in large part on the ascorbic acid content of the plasma. The normal activity of complement was contingent on its being present in the reduced state. Although other reducing agents such as glutathione, cysteine and hydrogen sulfide could bring about the reduction and activation, ascorbic acid was found to be the reducing agent of major importance in vivo.

Reedman and McHenry[36] have reported an extensive study of the degree of conjugation of ascorbic acid with protein in plants, but there has been no evidence that compounds of comparable stability occur in animal tissues.

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33. Green, D. E., and Richter, David: Adrenaline and Adrenochrome, Biochem. J. **31:** 596 (April) 1937.

34. Lemberg, R.; Cortis-Jones, B., and Norrie, M.: Coupled Oxidation of Ascorbic Acid and Hemochromogens, Biochem. J. **32:** 149 (Jan.) 1938.

35. Ecker. E. E.; Pillemer, L.; Wertheimer, D., and Gradis, H.: J. Immunol. **34:** 19 (Jan.) 1938.

36. Reedman, E. J., and McHenry, E. W.: Combined Ascorbic Acid in Plants, Biochem. J. **32:** 85 (Jan.) 1938.

CHAPTER XIX

THE PATHOLOGY OF VITAMIN C DEFICIENCY

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VALHALLA, N. Y.

The primary morphologic effects of vitamin C deficiency occur in the intercellular substances of certain mesenchymal derivatives. These can best be described in terms of the prototype of these structures, loose connective tissue. Under normal conditions the type cell, the fibroblast, lies in an amorphous ground substance within which fibrils (reticulum) are formed which may in turn become gathered into wavy bands of collagen. In this transformation the fibrils seem to become cemented together by a translucent matrix, the formation suggesting a colloid phenomenon, the setting of a gel. It is precisely this phase of the formation of intercellular materials which may be completely controlled by vitamin C. Thus in guinea pigs which have been depleted of vitamin C the ground substance and fibroblasts are present as in health but fibrils or collagen are not formed. When the deficiency is satisfied, translucent bundles and masses of collagenous materials reappear within eighteen hours. The formation of intercellular material of bone (osteoid tissue) and of teeth (dentin) may be similarly controlled by withholding or supplying vitamin C.

These changes have been accurately studied[1] and observed by various pathologists. The observations on experimental animals are in perfect harmony with the observations of spontaneous scurvy in man.[2] A point that is still disputed is whether the vitamin directly affects the intercellular colloids or the cells. Collagen may form in the absence of fibroblasts,[3] but in the primary response to treatment in scurvy the newly

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From the Laboratories of Grasslands Hospital.

[1] Wolbach, S. B., and Howe, P. R.: Intercellular Substances in Experimental Scorbutus, Arch. Path. **1:** 1 (Jan.) 1926.

[2] Aschoff, L., and Koch, W.: Skorbut: Eine pathologische-anatomische Studie, Jena, Gustav Fischer, 1919.

[3] Baitsell, G. A.: The Origin and Structure of a Fibrous Tissue Which Appears in Living Cultures of Adult Frog Tissues, J. Exper. Med. **21:** 455 (May) 1915.

formed material is seen in proximity to the fibroblasts or their processes.[4] While the cells are not significantly altered in appearance when the phenomena just described are taking place, they are altered during the late stages of chronic scurvy. Decision must be reserved on this point. Because the vitamin is assumed to have a pronounced effect on oxidation-reduction phenomena in the tissues and because hydrogen ion concentration is of critical importance to colloidal systems, it is tempting to accept the simpler explanation that the vitamin directly alters the physical character of the intercellular fluids.

Defective intercellular substances are seen in connective tissue, bones and teeth and are believed to occur in the blood vessels. An interesting modification of the process is apparent in partial deficiency, wherein a substitute material forms in the teeth in place of dentin. This has been spoken of as "osteodentin," since it resembles both materials. Similarly, in the bones, collagenous material appears in partial deficiency in place of osteoid. The tendency is for defective materials to form in connective tissue in partial depletion, and for nothing of a substantial nature to form in complete depletion. Further involution of the tissues in complete deficiency does not occur because the animals die.

Morphologic changes have not been detected in the capillaries and it is doubtful whether they occur. Capillaries originate from embryonic connective tissue and it is not surprising that they are affected. But where the weakness occurs is unknown. The endothelium of capillaries is believed to be fused together by a cement substance, but the capillary is also accompanied and surrounded by connective tissues and thin collagenous fibers ensheath the endothelium. Whether the effect of the deficiency is on the sheath or on the endothelial cement substance has never been decided. As in the case of the connective tissues the parenchymal cells appear to be normal and may multiply. Thus in young granulation tissue during depletion capillary budding has been seen; but this attempt at regeneration is soon aborted, presumably by lack of intercellular materials.[1]

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[4] Wolbach, S. B.: Controlled Formation of Collagen and Reticulum: A Study of the Source of Intercellular Substance in Recovery from Experimental Scorbutus. Am. J. Path. (supp.) **9**: 689, 1933.

The anatomic manifestations of scurvy are greatly modified by two factors, growth and stress. These are of such importance to the pathology of vitamin C deficiency that they must be discussed in some detail.

Growth so modifies the anatomic picture in scurvy that for 200 years the disease in children and infants was considered a variety of rickets, a condition which it superficially resembles more closely than it does adult scurvy. Hematomas become less and less frequent as the age of the patients increases. It is in the young, growing animal that bone pains occur; in the adult they are rare. Osteoporosis is greatly intensified in the young animal, just as it is always most pronounced at the particular part of the bone where growth is most active. Moreover, the most rapidly growing bones at the particular age at which scurvy occurs are most affected. This distribution and exaggeration of the lesions are similar to what occurs in rickets.

Stress modifies the site of the lesions and determines the extent and involvement of the various structures. Individual lesions can best be understood if the lines of force are borne in mind. Stress plays a major part in determining the site of the hemorrhages; the pressure of boots or a diaper, the location of a vessel near a bony prominence—these are the factors which determine whether or not hemorrhages will occur. The hemorrhages and other changes in muscles depend on stress.[5] In olden days it was noticed that blacksmiths and wool carders developed lesions in the shoulders and arms, soldiers in the calf and lumbosacral muscles.[6]

Lesions are indirectly modified by other factors, for example the presence or absence of a variety of other diseases (the list is long and rapidly growing and includes many infectious diseases, lobar pneumonia, typhoid and tuberculosis among others) and disturbances associated with an increased metabolic rate. These may operate similarly to growth and physical stress (activity) in determining the requirements of vitamin C and therefore, indirectly, the extent and degree of the scorbutic lesions. It would be instructive to determine whether the vitamin enters directly into inflammatory phenomena. There is substantial evidence to support the view that it is essential to the formation of blood cells,[7] including the granular leukocytes, and in this manner at least may be consumed at a higher than normal rate during infectious disease. Review of the larger series of cases of scurvy strongly encourages the belief that severe infectious diseases and scurvy go hand in hand and that each influences the severity and duration of the other.[8]

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[5] Dalldorf, Gilbert: The Lesions in the Skeletal Muscles in Experimental Scorbutus, J. Exper. Med. **50**: 293 (Sept.) 1929.

[6] Mahé, J.: Scorbut, in Dictionnaire encyclopédique des sciences médicales (Dechambre), series 3, **8**: 35, 1880.

If the influence of growth and stress is borne in mind, the individual lesions of scurvy may be described without note being taken of modifications and exceptions that may or may not occur because of these influences.

BONES

Skeletal lesions are commonest in the costochondral junctions, the distal end of the femur, the proximal end of the tibia, femur and wrist. The distal humerus and proximal ulna are usually spared. In the affected regions bone formation (enchondral bone growth) ceases and the existing osseous shell becomes rarefied, widened and conical. The epiphysis is not affected, at least in early stages, but may become displaced. False motion occurs in advanced cases and probably in even moderately severe cases in the costochondral junctions of the ribs. Microscopic examination reveals a rarefaction of the existing cortex, cessation of bone growth and replacement of the normal junction by a zone of collagen-poor connective tissue in which are embedded fragments of densely calcified cartilage matrix, bare of osteoid tissue. In controlled material it has been shown that the connective tissue cells of the marrow are migrated osteoblasts which have reverted to fibroblasts. The process suggests that in the absence of vitamin C the osteoblasts, unable to form osteoid tissue, revert to their prototype and attempt to form a fibrous union between diaphysis and epiphysis. This zone is generally spoken of as the "gerüstmark," or framework marrow, and in it lie the densely calcified fragments of cartilage matrix and bony trabeculae which are so conspicuous in roentgenograms. This is also the classic anatomic lesion of scurvy and may be found, in infants and children, in early or moderately advanced cases of the disease although frequently several bones must be searched.

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[7] Parsons, L. G., and Hawksley, J. C.: The Anhaematopoietic Anaemias, Arch. Dis. Childhood **8**: 117 (April) 1933.

[8] Nassau, E., and Singer, M. J.: Zur Kenntnis des Vorstadiums der Barlowschen Krankheit, Jahrb. f. Kinderh. **98**: 44, 1922.

Frequently this kind of lesion is complicated by hemorrhages, either large subperiosteal ones or small ecchymoses within or along the bone. In incompletely developed lesions, hemorrhages may be the deciding factor in an anatomic diagnosis.

If suspiciously enlarged costochondral junctions are submitted to microscopic examination, various stages of the typical scorbutic lesion will frequently be found. It is not necessary that it be fully developed. Experience rapidly recognizes a general fragility of the connective tissue fibers, a suggestive watery zone about the older osteoblasts and a defective osteoid tissue formation which plainly indicate the presence of scurvy. The greatest difficulties occur when scurvy is complicated by other diseases, for example rickets. It is at times difficult to distinguish scurvy from other dystrophies of bone such as osteogenesis imperfecta. Some of the latter conditions may indeed be related to the supply of vitamin C.

The periosteum shows a weakening of its attachment and a furious effort of connective tissue cells to reinforce it. This is most apparent at muscular attachments. Periosteal lesions of all degrees are prone to be complicated by hemorrhages due to the unyielding nature of the bone.

TEETH

In the teeth of adults with scurvy the dentin is seen to be resorbed and porotic. This process commences about Tomes's canals. The little replacement dentin that may be formed is inferior in appearance (osteodentin). In the pulp, chiefly about the vessels. atrophy, hyperemia, degeneration of the odontoblasts, the formation of small cysts and foci of calcification occur. The lesions develop first in the apex of the tooth and the bifurcation of the root canal. The fluid ground substance noted in experimental scurvy has also been observed in human material. The cementum is similarly affected.[9]

In the incisor teeth of the guinea pig early and extensive changes occur, because of the continuous rapid growth of these structures. In the guinea pig partial deficiency results in the formation of "osteodentin" and complete deficiency in undifferentiated matrix. In both cases the odontoblasts migrate from the predentin and revert to simpler types. Fish and Harris[10] demonstrated defects in enamel and cementum as well as in dentin.[11]

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[9] Westin, G.: Ueber Zahnveränderungen in Fällen von Skorbut bei Homo, Stockholm, Fahlcrantz, 1931.

GINGIVA

Lesions of the gingiva occur only when teeth are present and are most severe about deformed or broken teeth. The gums become swollen and boggy and bleed easily. In severe cases of scurvy they have become so large as to hide the teeth and make mastication painful and difficult. Rarefaction of the alveolar bones results in loosening of the teeth. The gingival lesions commence on the papillae, first as a hyperemia with dilated, thin walled vessels with a tendency to intractable hemorrhage. Disintegration of the epithelium follows and infection with ulceration, granulations and even gangrene. Lesions in the mouth are remarkably constant. Indeed, fifty years ago scurvy was defined as a generalized, afebrile disease accompanied by symptoms of fatigue, hemorrhages, usually starting on the legs, and changes in the gums.

MUSCLES

In severe scurvy, now rarely seen except in experimental animals, severe changes occur in the muscles. Fragmentation of the striated fibers and intense reparative efforts marked by multiplication of the sarcolemma occur. If the scurvy is prolonged, replacement by connective tissue poor in collagen may be seen. Höjer considered these lesions plus metastatic calcification characteristic of scurvy but they have since been reported[12] in various other experimental deficiencies (although without the calcification which is by no means constant in scurvy). If such muscle changes are accompanied by spontaneous hemorrhages they would seem to constitute strong evidence of the presence of scurvy, especially in man, since typical lesions of the kind have been reported in human cases of scurvy but not, to our knowledge, in other instances of human avitaminoses.

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[10] Fish, E. W., and Harris, L. J.: The Effects of Vitamin C Deficiency on Tooth Structure in Guinea Pigs, Brit. Dent. J. **58:** 3 (Jan. 1) 1935.

[11] The reader is referred to the report by Fish and Harris[10] for a divergent interpretation of the pathogenesis of scurvy as well as for an excellent description of the lesions in the teeth of guinea pigs. The view of Fish and Harris, similar to that advocated by Höjer, is that the primary effect of deficiency is an atrophy of the parenchymal cells. In my opinion this theory fails to explain the phenomena of complete deprivation already referred to and applies only to the late, nonspecific effects.

[12] Goettsch, M., and Pappenheimer, A. M.: Nutritional Muscular Dystrophy in the Guinea Pig and Rabbit, J. Exper. Med. **54:** 145 (Aug.) 1931. Wolbach, S. B.: The Pathologic Changes Resulting from Vitamin Deficiency, J. A. M. A. **108:** 7 (Jan. 2) 1937.

EYES

In severe scurvy, bloody tumors of the conjunctiva and ecchymoses of the eyelids and elsewhere about the eyes may appear. The other eye lesions that have been reported in the past are now known to be the results of simultaneous vitamin A deficiency (degeneration of the cornea, pigmentation and night blindness).

SKIN

The skin is of particular interest because of the frequency of signs of the disease there and the clue such easily observable lesions give to the general status of the patient's vitamin C supply. The lesion of the skin that is characteristic of scurvy is the perifollicular or petechial hemorrhage. This is commonest on the lower extremities or wherever pressure exposes the weakness of the capillaries. In the epidemics of a hundred years ago much was made not only of the presence of the perifollicular hemorrhage but also of changes in the follicle itself,[6] keratosis, dilated follicle mouth and fragmentation or loss of hair. Frequently dark, grayish pigmentation also was described. Identical observations were made during the World War.[2] As we now know, these were cases of combined deficiency of vitamins A and C. However, these observations raise the point whether similar perifollicular effects occur in uncomplicated scurvy, since it might otherwise seem that the presence of a diseased follicle, especially a thickened, hard one, would of itself predispose to hemorrhage. Undoubtedly similar lesions occur about normal hair follicles, as we have ourselves observed and other reports suggest, and have been absent in outspoken cases of vitamin A deficiency.[13]

OTHER LESIONS

Effusions, often blood stained, are common in the serous cavities. There may be slight edema about the ankles. Enlargement of the heart may occur. Circulatory collapse seems to have been a feature of the scurvy seen a hundred years ago and has been noted occasionally since. Whether there is a counterpart of the skeletal muscle lesions in the cardiac musculature, as Höjer believed, is doubtful. I have seen no such lesions, although fatty degeneration is common. Dilatation of the heart occurs. Atrophy of the bone marrow occurs in scurvy, and Wolbach[12] reports in experimental cases large areas of an amorphous material resembling amyloid. Except for this nothing characteristic has been reported, although the anemia of scurvy responds specifically to vitamin C.[14] The gastrointestinal tract is largely spared. Minute hemorrhages may be found and even larger ones with secondary necrosis or ulceration (in experimental scurvy),[15] but the study of human material does not support the view that the gastrointestinal tract suffers in a conspicuous fashion from vitamin C deficiency.

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[13] Frazier, C. N., and Hu, C. K.: Nature and Distribution According to Age of Cutaneous Manifestations of Vitamin A Deficiency, Arch. Dermat. & Syph. **33**: 825 (May) 1936.

The adrenals atrophy in chronic scurvy as the absorption of cortical fat and vitamin C progresses.[16] In the early stages they are swollen and hyperemic. The presence of vitamin C in the adrenal cortex may be easily demonstrated by soaking the split organ in silver nitrate, a procedure which is useful in the autopsy room.

Atrophy of the lymphatic tissue and to a lesser extent of other organs, especially glands of internal secretion, occurs but seems due to the general effects of debility.

CORRELATION OF LESIONS AND VITAMIN SUPPLY

It would be of considerable value to determine the various levels of vitamin C deficiency associated with these morbid manifestations, and to a certain extent this may be done. It is known, for example, that the first morbid effect of a severe depletion is capillary fragility[17] and probably also that the only morbid effect of a very slight negative vitamin C balance is capillary fragility or the presence of petechiae. Lind[18] himself said of the petechiae that they are the most constant symptom of all and occur even in the mildest cases, that they may indeed be the sole symptom and that in such cases their presence is sufficient for the diagnosis of scurvy. All recent work has served but to confirm this view. In human scurvy these changes are preceded only by a short prodrome of from five to eight days of weakness and lassitude. Petechiae then, of skin or nose or other cavities, are the first morbid expression of vitamin C deficiency and are related to what has recently been spoken of as subclinical scurvy. In this condition capillary fragility is present. Chemical tests reveal a subnormal vitamin C concentration in fasting blood samples. A state of unsaturation is evinced by measurements of the blood concentration and urinary excretion after a test dose of ascorbic acid.[19] The earliest stages of unsaturation are as a rule associated with morbid capillary resistance. Subclinical scurvy therefore appears to be definitely a pathologic state, albeit the deviation from health is slight. This degree of vitamin C deficiency is seldom recognized radiographically and with difficulty, if at all, by anatomic study. A comparable degree of deficiency in the guinea pig is uniformly associated with definite lesions in the incisors.

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[14] Mettier, S. R., and Chew, W. B.: The Anemia of Scurvy: Effect of Vitamin C Diet on Blood Formation in Experimental Scurvy of Guinea Pigs, J. Exper. Med. **55:** 971 (June) 1932. Höjer, J. A.: Studies in Scurvy, Acta pædiat. (supp.) **3:** 8, 1924.

[15] Smith, D. T., and McConkey, Mack: Peptic Ulcers (Gastric, Pyloric and Duodenal): Occurrence in Guinea Pigs Fed on a Diet Deficient in Vitamin C, Arch. Int. Med. **51:** 413 (March) 1933.

[16] Bessey, O. A.; Menten, M. L., and King, C. G.: Pathologic Changes in the Organs of Scorbutic Guinea Pigs, Proc. Soc. Exper. Biol. & Med. **31:** 455 (Jan.) 1934.

[17] Dalldorf, Gilbert: Criterion of Hemorrhagic Diathesis in Experimental Scurvy, J. Exper. Med. **53:** 289 (Feb.) 1931.

[18] Lind, J.: A Treatise of the Scurvy, ed. 3, London, S. Crowder, 1772.

In moderately advanced scurvy the chemical tests reveal distinctly abnormal values and considerable vitamin C is required to saturate the patient. In such cases the hemorrhages may be large, commonly subcutaneous ecchymoses or hematomas. It is a general rule in scurvy that the more severe the disease the more extensive the hemorrhages and the more likely is hemorrhage from larger vessels. In this degree of deficiency skeletal lesions are present and will be found if the search is thorough. The study of Park and his colleagues gives us a clue to the relative efficiency of methods of diagnosis. Of eighteen cases of scurvy in young children only two were diagnosed clinically and only six at routine postmortem examination, yet all had distinct lesions.[20]

L. J., and Kay, S. K.: Diagnosis of Vitamin C Subnutrition by Urinalysis, Lancet **1**: 71 (Jan. 12) 1935. Archer, H. E., and Graham, George: Some Observations on the Excretion of Ascorbic Acid, Lancet **1**: 710 (March 28) 1936. Abt, A. F.; Farmer. C. J., and Epstein, I. M.: Normal Cevitamic (Ascorbic) Acid Determinations in Blood Plasma and Their Relationship to Capillary Resistance, J. Ped. **8**: 1 (Jan.) 1936.

[20] Park, E. A.; Guild, H. G.; Jackson, D., and Bond, M.: The Recognition of Scurvy with Especial Reference to the Early X-Ray Changes, Arch. Dis. Childhood **10**: 265 (Aug.) 1935.

In severe scurvy nearly complete unsaturation may be demonstrated by chemical tests. The initial doses of vitamin, even when large, promptly are absorbed from the blood stream and little is lost in the urine. Hemorrhages are extensive and skeletal and gingival lesions outspoken.

CONCLUSIONS

It may safely be concluded therefore that the anatomic effects of vitamin C deficiency are very prompt to appear, certainly in the young, and that they occur, if the vascular changes are included, even in the mildest degrees of deficiency. Since clinical reports agree that subclinical scurvy, whether on the basis of chemical tests or measurements of capillary strength, is common, it may be assumed that morphologic stigmas due to the same deficiency are likewise common. However, both clinical and anatomic identification of scurvy remains, as it always has been, a matter of alertness on the part of the physician.

Finally, it may be pointed out that the recently acquired understanding of the scorbutic process affords pathologists and biologists a useful tool in the study of disturbances of intercellular materials. It should be of value also in the study of similar changes in senility.

CHAPTER XX

VITAMIN C

METHODS OF ASSAY AND DIETARY SOURCES

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The isolation, synthesis and development of practical methods of assay have given a great stimulus to the study of vitamin C.[1] More papers have appeared on methods of assay and distribution for vitamin C than for all the other vitamins combined. Therefore, no attempt is made here to present a systematic review of the literature. This has been done amply in the monograph by Sherman and Smith,[2] the yearly volumes of the *Annual Review of Biochemistry*,[3] the bulletin of the United States Department of Agriculture on "Vitamin Content of Foods"[4] and numerous reviews.[5] The facts and comments presented in this review may serve to give a general idea of the present state of the subject.

BIO-ASSAY

Chemical methods have rapidly replaced vitamin C determinations by bio-assay for many types of investigations. However, the more specific animal tests continue to be necessary in order to avoid the risk of misinterpretation of the chemical tests.

Guinea pigs kept on certain purified diets fail to gain weight, and the specific pathologic changes of scurvy develop. The degree of protection or cure of the deficiency bears a quantitative relation to the amount of

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From Harvard Dental School and the Department of Pathology, Harvard Medical School.

[1] Now named ascorbic acid or cevitamic acid.

[2] Sherman, H. C., and Smith, S. L.: The Vitamins, American Chemical Society Monograph, New York, Chemical Catalog Company, Inc., 1931.

[3] (*a*) Harris, L. J.: Vitamins, in Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press **1:** 357, 1932; **2:** 263, 1933; **3:** 264, 1934; **4:** 347, 1935. (*b*) von Euler, Hans: The Water Soluble Vitamins, ibid. **5:** 365, 1936. (*c*) Sherman, Caroline C., and Sherman, H. C.: The Vitamins, ibid. **6:** 346, 1937.

[4] Daniel, Esther P., and Munsell, Hazel E.: Vitamin Content of Foods, Miscellaneous Publication 275, U. S. Department of Agriculture, Bureau of Home Economics, June 1937.

[5] King.[23] Fellers.[68]

[6] Sherman and Smith.[2] Coward.[14] Coward and Kassner.[16]

the vitamin administered. This principle forms the basis for biologic methods of vitamin C analyses.

Preventive Method.[6]—The technic of Sherman and his associates,[2] built on the experience of earlier investigators as well as their own studies, has continued to be the most generally used for animal assay purposes. Briefly, the method is as follows: Young guinea pigs are fed ad libitum a basal vitamin C-free diet which theoretically supplies everything necessary for their normal nutrition except vitamin C. Negative control animals are kept on the basal diet only, while other animals are fed graduated amounts of the material to be tested. After from six to ten weeks the degree of protection is estimated by a standard postmortem appraisal of the extent of hemorrhage, the beading and enlargement of the costochondral junctions, the softness of the mandibles, scapulas and ribs, and the looseness of the incisors. An amount of test material just sufficient to afford complete protection from macroscopic scurvy is selected as a unit, and the results of all analyses are expressed in this unit.[7] With experience and by the use of the survival period, weight and degree of scurvy, one can estimate the degree of protection afforded by the lower test levels, thus bringing into a quantitative rating those animals which are not completely protected. The general uniformity of the test can now be improved by comparing the rate of growth and the intensity of the macroscopic lesions in the test animals with those in a similar group of animals which received graded levels of pure ascorbic acid.[8]

Curative Method.—Harris[9] and his associates have introduced a recovery method which is very useful. Less material is required and there is considerable saving in time and expense. Guinea pigs are kept on a basal diet as previously described, and after from two to three weeks animals are selected which show symptoms of scurvy and have begun to lose weight evenly. Graduated daily doses of the test material are then fed. After from ten to fourteen days the vitamin C value of the unknown is estimated by comparing the growth response with that obtained by feeding a like series of animals with graded amounts of pure ascorbic acid. Thus, with a continuous supply of guinea pigs acquiring scurvy, a test is completed in about two weeks. Care must be exercised in selecting animals for this test, because those which are "too sick" or which show signs of infection will not respond uniformly.

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[7] A Sherman unit, or a minimum protective dose, is equivalent to 0.5 to 0.6 mg. of ascorbic acid.

[8] International standards for assay purposes are obtained by application to the Protein and Nutrition Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, or to E. Fullerton Cook, Forty-Third Street and Woodland Avenue, Philadelphia.

[9] Harris, L. J.; Mills, J. I., and Innes, J. R. M.: The Chemical Identification of Vitamin C, Lancet **2**: 235 (July 30) 1932. Harris, L. J., and Ray, S. N.: Vitamin C in the Suprarenal Medulla, Biochem. J. **27**: 2006 (June) 1933.

Tooth Method.—Höjer[10] has proposed a method based on the observation that the degree of scurvy can be estimated by histologic examination of the teeth. A cross section of the root of a normal guinea pig incisor shows a row of long parallel odontoblasts surrounding the pulp and active in the formation of dentin. Höjer has described ten stages in the development of scurvy based on the amount of disorganization of the odontoblasts, the width, irregularity and structure of the dentin and the degree of calcification of the predentin. Key and Elphick[11] have described a modification in which, by the same criteria, they distinguish four degrees of scurvy. Using a large series of animals receiving graded doses of ascorbic acid, they plot the average degree of protection against the size of the dose, thus deriving a curve which shows the ratio of protection to dose at any level. When once established, this curve of reference may be used for calculations in successive assays, although it may be revised as additional data accumulate. An example will best show how this curve is used for a determination: A group of healthy growing guinea pigs (from 250 to 300 Gm.) were kept for a few days on a scorbutic diet plus greens. The test was started by withholding the greens. After three days one group of six animals received 0.9 mg. daily of ascorbic acid (International standard). Another group of six animals received 1 cc. of test material (orange juice) daily. Two guinea pigs were kept on the basal diet only (negative control animals). After fourteen days all the animals were killed and the lower jaws fixed and decalcified in 5 per cent trichloroacetic acid, embedded in celloidin and sectioned just anterior and parallel to the first molar at right angles to the longitudinal axis of the mandible. This method gives a cross section of the incisor root, which runs parallel with the jaw at this point.[12] Sections were stained with hematoxylin and eosin and with connective tissue stains. The degree of protection was scored from 1 to 4 for each tooth and the average obtained for all animals on a like level. The guinea pigs receiving the orange juice showed an average protection of 1.3, while those receiving the ascorbic acid averaged 1.8. On the basis of a curve previously prepared as described by Key,[13] the ratio of the protection in terms of ascorbic acid was found to be 0.62 divided by 0.90, which equals 0.69. Since, by definition, 1 mg. of ascorbic acid equals 20 international units, the potency of the orange juice equaled 13.8 international units per cubic centimeter. The curve of response is a device for calculating the relative potency of two materials which do not give an identical response.[14] The feeding of a standard with each assay makes possible a correction for differences in sensitivity of different stocks.[4]

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[10] Höjer, J. A.: Method for Determining the Antiscorbutic Value of Foodstuffs by Means of Histologic Examination of the Teeth of Young Guinea Pigs, Brit. J. Exper. Path. **7**: 356 (Dec.) 1926. Sherman and Smith.[2]

[11] Key, Kathleen, M., and Elphick, G. K.: A Quantitative Method for the Determination of Vitamin C, Biochem. J. **25**: 888 (March) 1931. Key and Morgan.[13]

Comparison of Methods.—The chief source of uncertainty in such quantitative methods lies in the unavoidable variation of individual experimental animals and in the indefinite nature of the end point. Complete protection from scurvy or a definite degree of partial protection are conditions difficult to judge. The answer to the problem of which is the best method of bio-assay for vitamin C depends on the experience of the investigator, equipment available, material to be tested, time available, purpose of the assay and many other factors. In addition to the economy of time, the tooth method or the curative method is most useful if the quantity of test material is limited or subject to change by storage. The specificity of the tooth method and the Sherman method is an important feature lacking from the growth response method of Harris. On the other hand, the two former methods are liable to cause subjective errors in judging the degree of scurvy. The need of histologic experience and special equipment for the tooth method are objections to its general use, while the other methods are adaptable to the usual laboratory equipment. It is my experience[15] and that of others[16] that a longer test period than that recommended by Harris for the protective method (e. g., four weeks) is necessary to obtain quantitative results comparable with those of other methods.

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[12] It is important that the section be made at a consistent level of the tooth, otherwise the degenerating odontoblasts which normally occur in the incisal part of the tooth may be confused with the scorbutic process. In a longitudinal section of a guinea pig tooth may be observed dental tissue in all stages of development. In the root the odontoblasts are young, regularly arranged and active in the formation of dentin; as they proceed up the persistently growing tooth, a normal sequence of senility and degeneration occurs. Near the biting end of the tooth the odontoblasts normally have much the same appearance as those which are present in the root in scurvy.

[13] Key, Kathleen M., and Morgan, Barbara G. E.: The Determination of the Vitamin C Value of Ascorbic Acid, Biochem. J. **27**: 1030 (April) 1933. Coward and Kassner.[16]

[14] Coward, Katherine H.: The Biological Standardization of Vitamins, Nutrition Abstr. & Rev. **4**: 705 (April) 1935. Daniel and Munsell.[4]

CHEMICAL METHODS

In general, chemical methods are more rapid and accurate than biologic methods but may lack specificity. However, when available and used with precaution they are of great help in many problems not subject to study by bio-assay.

The identification of vitamin C as a substance with striking reducing properties made it apparent that it had been studied previously as an unknown reducing substance in plant and animal tissues.[17] Tillmans[18] in studies of the freshness of natural foodstuffs had pointed out that a reducing substance is present in fresh fruits and vegetables which can be estimated by titration against an indicator, 2,6,dichlorophenolindophenol, and which appears to run parallel with their reported vitamin C content. The establishment of the identity of vitamin C by Waugh and King[19] stimulated experiments by Tillmans and his associates,[20] Harris and his associates[21] and Bessey and King[22] which showed the close correlation between the amounts of vitamin C as determined by biological assay and by titration with 2,6,dichlorophenolindophenol in many plant and animal tissues. Additional evidence has supported the view that the titration test when properly used is reasonably specific for vitamin C in natural products.[23]

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[15] Bessey, O. A.: Unpublished data.

[16] Coward, Katherine, and Kassner, Elsie W.: The Determination of Vitamin C by Means of Its Influence on the Body Weight of Guinea Pigs, Biochem. J. **30:** 1719 (Sept.) 1936.

[17] Szent-Györgyi, Albert: Observations on the Function of the Peroxadase Systems and the Chemistry of the Adrenal Cortex: Description of a New Carbohydrate Derivative, Biochem. J. **22:** 1387 (June) 1928. Harris, L. J.: Vitamins, in Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press **4:** 347, 1935. Tillmans, Hirsch and Hirsch.[18]

[18] Tillmans, J.; Hirsch, P., and Hirsch W.: The Reducing Property of Plant Foods and Its Relation to Vitamin C, Ztschr. f. Untersuch. d. Lebensmitt. **63:** 1 (Jan.) 1932.

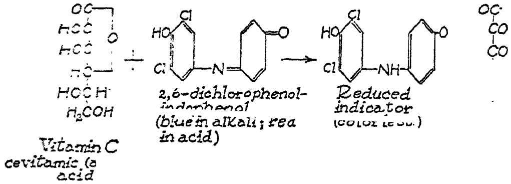
[19] Waugh, W. A., and King, C. G.: The Chemical Nature of Vitamin

Stanford University Press **1:** 357, 1932; **2:** 263, 1933.

[20] Tillmans, J.; Hirsch, P., and Vanbel, R.: The Reducing Value of Plant Tissues and Its Indication of Vitamin C, Ztschr. f. Untersuch. d. Lebensmitt. **65:** 145 (Feb.) 1933.

The essential features of the test are an extraction of the material under examination with an acid-precipitating agent to remove certain interfering substances and to stabilize and bring into solution the vitamin C and subsequent titration of the vitamin with a standardized indophenol solution.

The use of 2,6,dichlorophenolindophenol[22] to determine vitamin C is based on the fact that the colored indicator solution is quantitatively and rapidly reduced by vitamin C in acid solution to a colorless compound, as represented in the equation.



Reduction of indicator solution by vitamin C in acid solution.

Indophenol Titration Method.[24]—The indicator solution is prepared by dissolving the required amount of dry dye in hot water (50 mg. for food and 10 mg. for blood or urine per hundred cubic centimeters). Although the solid dye is stable, the aqueous solution slowly changes even when kept in the refrigerator and for this reason should be restandardized frequently. It is standardized against a carefully prepared solution of ascorbic acid containing 20 ± 0.1 mg. per hundred cubic centimeters of 3 per cent metaphosphoric acid.[25] A good commercial ascorbic acid is usually sufficiently pure for this purpose. However, the purity should be checked frequently by titration with iodine.[22] One should dilute 5 cc. of the aforementioned solution to 100 cc. with 3 per cent metaphosphoric acid for standardizing the more dilute dye solution. The strength of the reagent is expressed in milligrams of ascorbic acid per cubic centimeter of indophenol solution.

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[21] Harris, L. J., and Ray, S. N.: Specificity of Hexuronic (Ascorbic) Acid as Antiscorbutic Factor, Biochem. J. **27**: 580 (Feb.) 1933.

[22] Bessey, O. A., and King, C. G.: The Distribution of Vitamin C in Plant and Animal Tissues and Its Determination, J. Biol. Chem. **103**: 687 (Dec.) 1933.

[23] King, C. G.: Vitamin C, Ascorbic Acid, Physiol. Rev. **16**: 238 (April) 1936.

[24] Harris, L. J.: Vitamins, in Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press **3**: 264, 1934. Harris and Ray.[21] Bessey and King.[22] Musulin and King.[29]

Extracts of plant and animal tissues for vitamin C determinations are best obtained by thoroughly rupturing the cellular structures in metaphosphoric acid. The acid prevents the rapid oxidation of vitamin C due to enzymes [26] liberated by the macerated cells or by the presence of traces of certain metals.[27] In general, extracts of plant tissues can be prepared in this manner without detectable oxidation of the ascorbic acid. However, in many animal tissues, such as whole blood, liver, and kidney, unavoidable oxidation seems to occur during extraction.[15] Metaphosphoric acid [28] has replaced the use of other acid-extracting reagents because of its marked protective properties and its inertness toward the indicator. Trichloroacetic acid causes fading of the indicator, which may be very rapid with some batches.[29] Sulfuric acid reacts with the dye and should never be used.[15] Tungstic acid [30] has been used in blood determinations and, although it is a more complete protein precipitant, lacks the protective characteristics of metaphosphoric acid.

An extract of a representative portion of the plant or animal tissue under examination (from 5 to 10 Gm.) is prepared by grinding in a small mortar with sand and fresh 3 per cent metaphosphoric acid until a thin paste is formed. The solids are thrown down in a centrifuge and the clear extract is decanted. Another portion of extracting liquid is used to wash the mortar and is stirred into the solids, which are again centrifuged. The rinsing and washing process is repeated, and the decanted aliquots of the extracts are diluted to 50 cc. Aliquots (10 cc.) of the extracts are titrated with the standard 2,6,dichlorophenolindophenol solution until a faint pink end point is reached. A 10 cc. microburet graduated in 0.01 cc. is convenient.

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[25] Metaphosphoric acid solution is slowly converted to orthophosphoric acid. It should be prepared fresh frequently and should be kept in the refrigerator.

[26] Kertesz, Z. I.; Dearborn R. B., and Mack, G. L.: Vitamin C in Vegetables: IV. Ascorbic Acid Oxidase, J. Biol. Chem. **116:** 717 (Dec.) 1936. Johnson, S. W., and Zilva, S. S.: The Oxidation of l-Ascorbic Acid by Plant Enzymes, Biochem. J. **31:** 438 (March) 1937.

[27] Barron, E. S. G.; DeMeio, R. H., and Klemperer, Friedrich: Biological Oxidation: V. Copper and Hemochromogens as Catalysts for the Oxidation of Ascorbic Acid, J. Biol. Chem. **112:** 625 (Jan.) 1936.

[28] Fujita, Akiji, and Iwatake, Danzo: The Determination of Vitamin C with 2,6-Dichlorophenolindophenol, Biochem. Ztschr. **277:** 293, 1935.

[29] Musulin, R. R., and King, C. G.: Metaphosphoric Acid in the Extraction and Titration of Vitamin C, J. Biol. Chem. **116:** 409 (Nov.) 1936.

[30] Taylor, F. H. L.; Chase, D., and Faulkner, J. M.: The Estimation of Reduced Ascorbic Acid in Blood Serum and Plasma, Biochem. J. **30:** 1119 (July) 1936.

The indophenol reacts rapidly with vitamin C and should be dropped in at a brisk rate. The end point for most plant tissues is stable for twenty seconds or more, while animal tissues usually fade after from ten to fifteen seconds because of a slow reaction by other substances. Such extracts should be titrated only to an end point at which rapid fading ceases. A correction for the amount of dye required to bring 10 cc. of the extracting acid to the same end point should be applied to all determinations, including the standardizations. This quantity is greater than the usual indicator corrections and should never be neglected. The results are most conveniently expressed in terms of milligrams of ascorbic acid per hundred grams of fresh weight, but in studying vitamin C in tissues which vary considerably in water content, one may get a clearer picture by basing the analysis on dry weight.

Comment.—Since the indophenol method depends on the reduction of the reagent by vitamin C, any substance having a reducing potential lower than the dye is a possible source of interference. However, most such substances found in natural systems react with the indicator at a perceptibly slower rate than ascorbic acid under the conditions of the titration. This is a requirement for the specificity and accuracy of the method. The end point with plant tissues is stable for some time, but many animal tissue extracts and urines contain sufficient amounts of slow-reducing substances to make the end point less distinct. This is especially true when small quantities of ascorbic acid are being titrated. In such cases the accuracy of the titration depends on the skill of the observer in detecting the point at which rapid reduction is replaced by a slow fading of the indicator. It is true that this feature limits the accuracy of the method, but the precision in most cases is still within the range of biologic variation. The errors are of about the same order as those for blood sugar tests. Unknown reducing materials are reported to interfere with the test in nerve tissue,[31] eye lens and aqueous humor,[32] the liver of some species[33] and tumor tissues.[34] Reduction by phenols, tannins, glutathione and many other substances is eliminated by titration in acid solutions below p_H 3. Cysteine, sometimes present in autolyzed tissue, thiosulphate and ergothionine must be estimated separately or removed from the system. Ergothionine, thiosulfate, cysteine and other organic sulfur compounds may be removed by a rather unpractical mercuric acetate treatment suggested by Emmerie.[35] Reductone (glucic acid),[36] redutic acid[37] and other derivatives formed in hot alkaline sugar solutions reduce the dye in a manner indistinguishable from that of vitamin C. Similar compounds are found in yeast, mold, malt extract and beer.[38] These examples illustrate the point that when one is dealing with a type of material not previously checked it is best to establish the validity of the test by other means.

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31. Young, F. G., and Mitolo, M.: A Reducing Substance in Brain Tissue, Nature **133**: 572 (April) 1934. Plaut, F.; Bülow, M., and Pruckner, F.: Chemical and Spectroscopic Comparison of the Vitamin C Content of Urine, Spinal Fluid and Serum, Ztschr. f. physiol. Chem. **234**: 131 (April) 1935.

32. Birch, T. W., and Dann, W. J.: Ascorbic Acid in Eye Lens and Aqueous Humor of the Ox, Biochem. J. **28**: 638, 1934. von Euler, Hans, and Malmberg, Maj.: Antiscorbutic Action of the Eye Lens and Its Contents of Reductone and Sulphydryl Compounds, Ztschr. f. physiol. Chem. **230**: 225, 1934.

33. Borsook, Henry; Davenport, H. W.; Jeffreys, C. E. P., and Warner, R. C.: The Oxidation of Ascorbic Acid and Its Reduction in Vitro and in Vivo, J. Biol. Chem. **117**: 237 (Jan.) 1937.

34. Kellie, A. E., and Zilva, S. S.: The Identity of the Indophenol Reducing Substance in the Jansen Rat Sarcoma, Biochem J. **30**: 1216 (July) 1936. Harris, L. J.: A Reducing Substance in Tumor, Nature **132**: 605 (Oct.) 1933. Harris.[24]

35. Emmerie, Adrianus: Separation of Cysteine from Ascorbic Acid by Mercuric Acetate, Biochem. J. **28**: 268, 1934. Emmerie, Adrianus, and Van Eekelen, Marie: The Chemical Determination of Vitamin C with Removal of Interfering Reducing and Colored Substance, ibid. **28**: 1153, 1934; Some Critical Remarks on the Determination of Ascorbic Acid, ibid. **30**: 25 (Jan.) 1936.

36. Nelson, E. K., and Browne, C. A.: The Properties and Chemical Constitution of Glucic Acid, J. Am. Chem. Soc. **51**: 830 (March) 1929. von Euler, Hans, and Martius, Carl: Reductone and Ascorbic Acid, Ann. d. Chem. **505**: 73, 1933. King.[23] Nelson and Mottern.[79]

37. Reichstein, T., and Oppenhauer, R.: Reductic Acid: A Strong Reducing Split Product of Carbohydrates, Helvet. chim. Acta. **17**: 390, 1934.

38. Harris, L. J.: Chemical Tests for Vitamin C and the Reducing Substances Present in Tumor and Other Tissues, Nature **132**: 27 (July) 1933. von Euler.[3] Fox and Stone.[49]

Recent evidence[38a] suggests that there may exist in certain plant tissues significant quantities of physiologically available combined ascorbic acid which is not extracted by the usual acid extracting reagents and therefore is not included in the titration value. However, by use of the more accurate photoelectric indophenol method[38b] the author has been unable to confirm this observation. The titration figures of Reedman and McHenry may have another interpretation. Unless the extracts are carefully prepared and titrated without too much delay, oxidation of vitamin C by liberated enzymes or traces of metals may cause a low titration figure. If the oxidation has proceeded beyond the first step as previously indicated,[39] both the chemical and the biologic value will be low. In general, reversibly oxidized ascorbic acid (dehydroascorbic acid) represents an insignificant portion of the total biologic value of fresh or storage food products.

The titration value of some tissue extracts may be increased by preliminary treatment with hydrogen sulfide.[35] Some investigators have interpreted this increase as due to reduction of dehydroascorbic acid and have modified the method to include both forms of ascorbic acid.[39a] There is some doubt as to how much of this increase is due to the reduction of dehydroascorbic acid and how much to the reduction of other substances which are then included in the titration. The uncertainty of the procedure is also increased by the necessity of completely removing the gas because hydrogen sulfide also reduces the indicator. Emmerie and Van Eekelen recommended the use of mercuric acetate to remove cysteine ergothionine and of thiosulfate followed by treatment with hydrogen sulfide. The extra manipulation involved, with uncertainty at every step, makes the method at present of doubtful value.

Microtitration with indophenol for special purposes has been described by Birch, Harris and Ray,[40] Farmer

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38a. Reedman, E. J., and McHenry, E. W.: Combined Ascorbic Acid in Plant Tissues, Biochem. J. **32:** 85 (Jan.) 1938.

38b. Bessey, Otto A.: A Method for the Determination of Small Quantities of Ascorbic Acid and Dehydroascorbic Acid in Turbid and Colored Solutions in the Presence of Other Reducing Substances, J. Biol. Chem. **126:** (Dec.) 1938.

39. Borsook and his co-workers.[33] King.[59]

39a. Kertesz, Z. I.; Dearborn, R. B., and Mack, G. L.: Vitamin C in Vegetables. IV. Ascorbic Acid Oxidase, J. Biol. Chem. **116:** 717 (Dec.) 1936. Reedman.[38a] Bessey.[38b] Bursook.[33]

40. Birch, T. W.; Harris, L. J., and Ray, S. N.: A Microchemical Method for Determining the Hexuronic Acid (Vitamin C) Content of Foodstuffs, Biochem. J. **27:** 590, 1933.

and Abt[41] and Glick.[42] The last named has applied the Linderstrom technic to microtome-cut sections of glandular tissues with some success.

Photoelectric Indophenol Method.[42a]—Although the titration procedure has been very useful and quite satisfactory for most plant and some animal tissues, there are several limitations and hazards involved in its general use as indicated above. The process of titration, with usual end-point detection, is not precise for the determination of small quantities of ascorbic acid. It has been impossible to satisfactorily analyze extracts which are highly colored, such as those from beets, berries, and certain leaves. Turbid solutions lead to high end-points. The most accurate method for vitamin C determination, so far suggested, one which is suited to general use and which overcomes most of the objections and limitations of the titration technique, is the photoelectric indophenol method. Mindlin and Butler[57] have recently applied this procedure to the analysis of blood plasma. The author[38b] has improved and developed the method for general use. In this method the decrease in concentration of colored indicator produced by the addition of an amount of tissue extract insufficient to cause complete reduction of the dye is measured by means of a photoelectric colorimeter. This eliminates the error of subjective detection of the end-point. The precision is superior to other methods with samples containing 5 to 20 micrograms of ascorbic acid even in the presence of pigments and turbidity. An accurately standardized dye is not needed. Observations of the rate of fading of the color with time permits the detection and estimation of reducing substances reacting at a slower rate than ascorbic acid. The objection to the general use of this method is the need of a special instrument.

Other Chemical Methods.—Alternative methods based on the reduction of ferrocyanide,[43] phosphotungstic

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41. Farmer, C. J., and Abt, A. F.: Determination of Reduced Ascorbic Acid in Small Amounts of Blood, Proc. Soc. Exper. Biol. & Med. **34:** 146 (March) 1936.

42. Glick, David, and Biskind, G. R.: The Histochemistry of the Adrenal Gland: I. The Quantitative Distribution of Vitamin C, J. Biol. Chem. **110:** 1 (June) 1935; The Histochemistry of the Hypophysis Cerebri: The Quantitative Distribution of Vitamin C, ibid. **110:** 583 (Aug.) 1935.

42a. Mindlin.[57] Bessey.[38a]

43. Tauber, Henry, and Kleiner, I. S.: A Method for the Quantitative Determination of Ascorbic Acid (Vitamin C), J. Biol. Chem. **108:** 563 (Feb.) 1935.

acid,[44] methylene blue[45] phosphomolybdic acid[46] and other color reagents have been proposed. From a theoretical standpoint it would seem that these reagents might be as specific and useful as indophenol, but for one reason or another they have not been widely adopted. In some cases they react too slowly in acid solutions (p_H from 2 to 3 is a necessary condition for specificity). The color changes often vary with time. Many of these reagents react quantitatively with pure ascorbic acid solution but are inhibited or reduced by other materials in urine and tissue extracts. Ascorbic acid in some cases shows side reactions with the reagent, thus interfering with quantitative reduction. A spectroscopic method[47] in which the intensity of absorption (265 millimicrons) is measured before and after the destruction of the vitamin has been of value in checking the accuracy of the indophenol method. Extracts require special attention in removing irrelevant absorbing materials. Ball has used oxidation-reduction curves as a method for determining both the oxidized and reduced forms of ascorbic acid in certain special systems.[47a] Tauber and Kleiner[48] advocated the determination of reducing capacity before and after the destruction of the vitamin by an oxidase as a means of increasing the specificity of the test. This method is also not entirely specific, because of the nonspecificity of the enzyme.[49] Roe[50] has described a method based on another principle, the color reaction between aniline acetate and furfural, a decomposition product of ascorbic acid.

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[44] Medes, Grace: The Determination of Ascorbic Acid in Urine with Phospho-18-Tungstic Acid, J. Biochem. **29:** 2251 (Oct.) 1935.

[45] Gál, Imre: Estimation of Ascorbic Acid by Titration, Nature **138:** 799 (Nov.) 1936.

[46] Bezssonoff, N.: Sur l'identification de la vitamine C, Bull. Soc. chim. Biol. **16:** 1107 (July, Aug.) 1934. Bezssonoff, N.; DeLire, A., and Van Wien, H.: L'activité réductrice de la vitamine C et d'autres réducteurs biologiques exprimée en fonction de leur concentration, ibid., p. 1133. Bezssonoff, N., and Van Wien, H.: Sur l'identification et le dosage de la vitamine C dans les milieux biologiques, ibid., p. 1160. von Euler, Hans, and Burstrom, Dagmar: Ascorbic Acid Determinations in Urine by Titration, Biochem. Ztschr. **283:** 153, 1936.

[47] Robertson, E. B.: Spectrophotometric Estimation of Ascorbic Acid, J. Soc. Chem. Industry **53:** 277 (March) 1934. Chevallier, André, and Choron, Yvonne: Spectrophotometric Method for Estimating Vitamin C in Tissues, Compt. rend. Soc. de biol. **124:** 453, 1937.

[47a] Ball, E. G.: Studies on Oxidation-Reduction. XXIII. Ascorbic Acid, J. Biol. Chem. **118:** 219 (Mar.) 1937.

[48] Tauber, Henry, and Kleiner, I. S.: An Enzymic Method for the Estimation of True Vitamin C, J. Biol. Chem. **110:** 559 (Aug.) 1935.

[49] Fox, F. W., and Stone, William: Specificity of Indophenol in the Estimation of Ascorbic Acid in Fermented Products, Nature **140:** 234 (Aug.) 1937. Johnson and Zilva.[26] King.[50]

[50] Roe, J. H.: The Determination of Ascorbic Acid as Furfural and a Comparison of Results Obtained by This Method and by Indophenol Titration, J. Biol. Chem. **116:** 609 (Dec.) 1936.

In general the special features of these procedures are outweighed by features which make them less straightforward and less specific than the indophenol method. A discussion of the weaknesses and merits of all the proposed chemical methods is not within the realm of this paper.

Urine Technic.[51]—The excretion of vitamin C in the urine of normal man depends primarily on the relative degree of saturation of the tissues and the immediate intake of ascorbic acid. As a state of tissue saturation is approached, the quantity of ascorbic acid excreted in the urine gradually increases until at saturation a marked abrupt elevation occurs. The quantity of ascorbic acid required to induce saturation appears to be a measure of the state of vitamin C nutrition of the patient.[3c]

The analytic procedure for urine is essentially the same as that previously described for tissues. Each specimen of voided urine is measured and a 50 cc. volume containing 10 cc. of 20 per cent metaphosphoric acid is filled to the mark and stoppered. Aliquots should be stored in the refrigerator and titrated within eight to ten hours, during which time relatively little loss occurs. For analysis, the specimen is centrifuged if cloudy, and from 2 to 5 cc., depending on the concentration of ascorbic acid, is diluted to 10 cc. with 3 per cent metaphosphoric acid. The mixture is quickly titrated to a definite pink end point against a standard indophenol solution, a microburet being used. After correction for the titration blank and the added metaphosphoric acid, the total excretion of the specimen is estimated and the twenty-four hour output calculated. The reducing capacity of the urine collected during a preliminary period before the test establishes the excretion level of the patient before treatment with ascorbic acid. This also places the interpretation of the test on a comparative basis, thus correcting for the small constant effect of reducing substances other than ascorbic acid usually present in the urine.[52] Heinemann [53] has reported a significant interference by thiosulfate, especially when the diet is high in protein. However, the virtual disappearance of the reducing value in cases of scurvy and extreme deficiency and its restoration at a predicted rate when ascorbic acid is fed contribute strong evidence for the accuracy of the test. Provided manipulations are carried out properly and interpretations of too fine a character are not attempted, the method is of real value.

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[51] Sendroy, Julius, Jr., and Schultz, M. P.: Studies of Ascorbic Acid and Rheumatic Fever: I. Quantitative Index of Ascorbic Acid Utilization in Human Beings and Its Application to the Study of Rheumatic Fever, J. Clin. Investigation **15**: 369 (July) 1936. Harris, L. J., and Ray, S. N.: Diagnosis of Vitamin C Subnutrition by Urine Analysis, Lancet **1**: 71 (Jan. 12) 1935. Abbasy, M. A.; Harris, L. J.; Ray, S. N., and Marrack, J. R.: Diagnosis of Vitamin-C Subnutrition by Urine Analysis, Lancet **2**: 1399 (Dec. 21) 1935. von Euler.[3] Sherman and Sherman.[3]

The estimation of the excretion of ascorbic acid before and after a single dose is also of value in demonstrating the presence of subnutrition of vitamin C.[53a] This procedure undoubtedly shows the absence of saturation, but sufficient data have not been collected to allow for an estimation of the degree of deficiency such as is possible by the repeated dose method. The use of the photoelectric technique [36b] for urine, eliminates the interference from color and slow-reducing substances and improves the precision of the analysis.

Blood Technic.[54]—The vitamin C level of normal blood plasma as estimated by titration shows a close correlation with the level of intake, thus providing means for evaluating the reserve vitamin C by a single determination for persons in that wide zone between scurvy and saturation (from 0.3 to 1.8 mg. per hundred cubic centimeters). This rapid direct application of the test will undoubtedly replace the less practical urine technic for many purposes.

The indophenol titration method modified to the extent of changing the concentration of the dye, the size of the aliquot and the type of burette has been applied to blood plasma. With slight variations, the procedure that has been generally used is as follows. Five or more cubic centimeters of fasting blood is drawn, oxalated and centrifuged in the usual manner. Two cc. of the plasma is pipetted immediately into 8 cc. of 3 per cent metaphosphoric acid solution, the mixture stirred, stoppered and centrifuged. A 5 cc. aliquot (equal to 1 cc. plasma) of the clear centrifugate is titrated as previously described with dilute 2, 6, dichlorophenol-indophenol. After a correction for the blank which always should be performed parallel with a determination, the ascorbic acid is calculated and expressed as milligrams per hundred cubic centimeters (milligram per cent) of plasma. Any substance added to the blood plasma (i. e., oxalate, cyanide) must be included, as usual, in the blank. In competent hands, with all sources of error considered, this titration is probably within 0.1 to 0.2 mg. of the true ascorbic acid content of the blood for the range 0.3 to 1.8 mg. per hundred cubic centimeters. In many laboratories, potassium cyanide (2 to 4 mg. per 5 cc.) has been added to the freshly drawn blood to retard the slow oxidation which may occur in either plasma or filtrate when traces of copper or other catalyst are present. In some localities, unless the glassware and reagents receive special attention, traces of metals may cause significant errors.[55] However, when carefully prepared materials are used, cyanide is unnecessary, for under these conditions, properly drawn plasma is stable for at least an hour and the filtrates do not change for several hours.[55a] Recent experience indicates other reasons why the general use of cyanide should be discontinued. If this reagent is improperly used at high concentrations or if an impure specimen is used, errors may result depending upon the magnitude of these factors.[55b] However, if a good quality cyanide has been used at the proper concentration, no significant errors result [55a] and blood values determined under these conditions are not to be discredited, as suggested by some writers.[55c] Metaphosphoric acid is preferred as a deproteinizing agent for reasons previously stated. Plasma or serum is preferred to whole blood for vitamin C determinations because of the greater stability of the filtrates.[56] Substances liberated from red blood cells immediately inactivate ascorbic acid even in the presence of cyanide and the acid. This is one reason why plasma which shows evidence of hemolysis is unsuitable for ascorbic acid determination. Also, substances which cause a slow fading of the dye are liberated from red cells, thus tending to obscure the end-point.[15] If whole blood is to be used, it is necessary to treat the filtrate with mercuric acetate as previously mentioned, followed by H_2S treatment.[56a] This titration is at present unsatisfactory because substances other than ascorbic acid, not eliminated by the mercury, also reduce the dye, leading to end-point difficulties. At present the most satisfactory method for blood vitamin C determinations is the macrophotolectric indophenol technique for plasma previously described.[57]

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[52] Borsook and his co-workers.[33] Heinemann.[53]

[53] Heinemann, M.: Dietary Influence on the Amount of Ascorbic Acid and Other Reducing Substances in Urine, Acta brev. Neerland. **6:** 67 (Aug.) 1936.

[53a] Ralli, Elaine P., and Friedman, G. J.: The Response to the Feeding of Cevitamic Acid in Normal and Deficient Subjects as Measured by a Vitamin C Excretory Test, Ann. Int. Med. **11:** 1196 (May) 1938.

[54] Abt, A. F.; Farmer, C. J., and Epstein, I. M.: Normal Cevitamic (Ascorbic Acid) Determinations in Blood Plasma and Their Relationship to Capillary Resistance, J. Pediat. **8:** 1 (Jan.) 1936. Ingalls, T. H.: Studies on the Urinary Excretion and Blood Concentration of Ascorbic Acid in Infantile Scurvy, J. Pediat. **10:** 577 (May) 1937. Sherman and Sherman.[3] Bessey.[15] Farmer and Abt.[41] Pijoan and Klemperer.[55]

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[55] Pijoan, Michel, and Klemperer, Friedrich: Determination of Blood Ascorbic Acid, J. Clin. Investigation **16:** 443 (May) 1937.

[55a] Friedman, G. J.: Rubin, S. H., and Kees, Walter: Effects of Addition of KCN to Whole Blood on Indophenol-Reducing Power of Plasma, Proc. Soc. Exper. Biol. & Med. **38:** 358 (April) 1938. Bessey.[15] Butler.[55b]

[55b] Butler, A. M.: Private communications.

[55c] Farmer, C. J., and Abt, A. F.: The Invalidation of Plasma Ascorbic Acid Values by Use of Potassium Cyanide, Proc. Soc. Exper. Biol. & Med. **38:** 399 (April) 1938.

[56] Borsook and his co-workers.[33] Farmer and Abt.[41]

Mindlin and Butler [57] also have described a microphotoelectric method which yields results satisfactory for clinical purposes and which requires only 0.2 cc. of capillary blood, thus obviating the need for a venous puncture. However, like most micro techniques, the method should be used only when circumstances prevent the use of the macro method. Titration procedures for small amounts of blood plasma have been described.[41] Determinations of such small quantities of ascorbic acid by a titration technique are subject to so many errors that in most cases it cannot be used with confidence.

It is important to emphasize the fact that titrations of blood and many other tissues which are extremely low in ascorbic acid have a limited accuracy even when done with the greatest care. A disregard for this fact is apparent from published investigations. Conclusions based on differences far smaller than the errors of the method used are obviously of doubtful value. Such misinterpretation can be avoided only by a thorough understanding and proper application of the technic.

DIETARY SOURCES

This discussion is concerned with the distribution of vitamin C in food and necessarily lays emphasis on this subject. It should be remembered that adequate nutrition is a problem of supplying many factors in proper proportions and can be met only by use of a variety of foods. Condemnation of a food product because it possesses a low vitamin C value, unless this is a consequence of improper processing or handling, is unjustified. Some foods with many excellent nutritional properties are naturally of low antiscorbutic value.

[56a] Heinemann, Martin: Requirements for Vitamin C in Man, J. Clin. Invest. **17**: 671 (Sept.) 1938.

[57] Mindlin, R. L., and Butler, A. M.: The Determination of Ascorbic Acid in Plasma: A Macro and Micro Method, J. Biol. Chem. **122**: 673 (Feb.) 1938.

Ascorbic acid value as far as nutrition is concerned is dependent primarily on two factors: (1) the natural potency of the particular plant or animal tissue and (2) the consequence of aging and processing which occurs before its use as food.

Among the richest sources of vitamin C are oranges, lemons, grapefruit (raw or canned), tangerines, tomatoes (raw or canned) fresh strawberries, green peppers and raw cabbage.[58]

Properly prepared green leafy vegetables have good vitamin C value in spite of the relatively large losses from cooking or canning. In this group may be classed cabbage, spinach, brussels sprouts, kale, broccoli and vegetable greens, such as turnip, beet and dandelion.

Apples, bananas, pineapples (canned), potatoes, green beans and green peas, although containing less antiscorbutic activity, are important sources because of the quantities included in the diet.

The lettuce group, including escarole, have a lower vitamin C content than is generally realized but, since they are usually consumed raw, compare favorably with cooked vegetables. Other important vitamin C-containing foods are kohlrabi, onions, turnips, parsnips and cauliflower.

Dry cereals and legumes of all varieties are devoid of vitamin C, but almost any seed soaked in water for twenty-four hours and kept moist for a few days until it sprouts becomes an effective antiscorbutic substance even when cooked. The British committee on accessory food factors has recommended the use of sprouting seeds as antiscorbutic foods in times of famine.

Cooked muscle meat[2] is practically free from ascorbic acid, although experience seems to show that if consumed rare or raw in large quantities it prevents scurvy. Liver is greatly superior to muscle in this respect. The distribution of vitamin C in other animal

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58. Sherman and Smith.[2] Daniel and Munsell.[4] Fellers.[63] Sherman.[83]

tissues (not important as food) has been discussed in a previous paper of this series.[59]

Butter, eggs and cheese contain no vitamin C. The vitamin C value of commercial pasteurized milk is very small.

Variety.[60]—Before the introduction of the chemical method, it was not practical to undertake any comprehensive study of the influence of various factors such as variety, type of soil, season and maturity on the vitamin C potency of fruits and vegetables. Enough information has been obtained now to indicate clearly the need of attention to these points in attempting to obtain food of the highest antiscorbutic value.

Certain varieties of tomatoes [61] contain double the quantity of vitamin C of others grown on the same soil under the same conditions. Canners of tomato juice should give as much attention to this fact as to methods of processing in order to provide the most potent product. Some varieties of apples [62] are definitely higher in antiscorbutic value than other varieties. Similar consistent differences according to variety have been reported for spinach,[63] cabbage,[64] beans and peas [65] and probably exist for most fruits and vegetables.

Soil conditions affect the vitamin C content of spinach. Several varieties grown on upland soil were found to average 50 per cent richer than the same varieties grown on muck soil when compared under otherwise similar conditions.[66] Fall spinach when compared with the same varieties grown in the spring was found to be appreciably higher in ascorbic acid content. It therefore seems likely that crops from year to year may vary in ascorbic acid value because of conditions such as moisture and temperature.

Maturity and Origin.—Tomatoes increase somewhat in vitamin C content as the fruit ripens.[67] However,

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59. King, C. G.: Vitamin C Chemistry, J. A. M. A., to be published.

60. Daniel and Munsell.[4] Fellers.[68] Tressler, Mack and King.[73]

61. Tripp, Francis; Satterfield, G. H., and Holmes, A. D.: Varietal Differences in the Vitamin C (Ascorbic Acid) Content of Tomatoes, J. Home Econ. **29**: 258 (April) 1937. Maclinn, Fellers and Buck.[77]

62. Smith, G. G., and Fellers, C. R.: Vitamin C Content of Twenty-One Massachusetts Grown Varieties of Apples, Proc. Am. Soc. Horticultural Sc. **31**: 89, 1934. Batchelder.[76]

63. Tressler, D. K.; Mack, G. L., and King, C. G.: Vitamin C Content of Vegetables: I. Spinach, Food Research **1**: 3 (Feb.) 1936.

64. Gould, Stella; Tressler, D. K., and King, C. G.: Vitamin C Content of Vegetables: V. Cabbage, Food Research **1**: 427 (Oct.) 1936.

65. Mack, Tressler and King, footnotes 72 and 75.

66. von Hahn, F. V., and Gorbling, J.: Influence of Fertilizer on the Vitamin C Content of Spinach, Ztschr. f. Untersuch. Lebensmitt. **65**: 601 (April-June) 1933. Tressler, Mack and King.[63]

67. Sherman and Smith.[2] Tripp, Satterfield and Holmes.[61]

mature green tomatoes may have nearly the same value as the red ripe fruit.[68] The ascorbic acid content of green peppers increases considerably on reddening. A single fruit which is green on one side and red on the other may show a difference of as much as 75 per cent.[69] A lower vitamin C content in oranges sprayed with lead arsenate has been reported.[70] Immature Bramley seedling apples are as rich in vitamin C as mature fruit.[68] Most green leafy and root vegetables contain the greatest concentration of vitamin C during the period of most rapid growth. Sweet corn [71] and green peas [72] show a maximum vitamin C content during the tender stage, and the content decreases as the seed matures. This is consistent with the lack of vitamin C in nonrespiring mature seeds. Ethylene ripening itself has no effect but tends to encourage early picking and may lead to a slightly lower vitamin C value due to immaturity.[73]

How and from what most plants and some animals form ascorbic acid is not clear, but the distribution in animal tissues shows it to occur in the greatest concentration in the most actively functioning tissues, in the adrenal cortex, corpus luteum, thyroid, pituitary body and liver. This distribution proves to be true also in leaves and growing shoots and suggests a fundamental concern with respiratory processes. However, many rapidly growing fruits are low in ascorbic acid, and the respiratory activity of lemons, oranges and tomatoes does not seem to be unusually high, although these are among the richest sources of ascorbic acid. Eggs and seeds are devoid of vitamin C, but the vitamin forms immediately from unknown constituents when a chick embryo develops or the seed sprouts.

Storage.[68]—Time, temperature, care in handling and type of product are important factors in the problem of the stability of vitamin C during storage. Spinach which has stood for a few days at room temperature and has become wilted loses about one half of its ascorbic acid, while the same spinach kept at 37 or 38 F. for the same period retains its original value.[74] This is true in general of green leafy vegetables of this type. Cabbage stored at room temperature loses about 25 per cent of its vitamin C in a month, while at 45 or 50 F. the loss is only 10 per cent.[64] Green beans and peas, like spinach, rapidly lose their ascorbic acid at room temperature but keep well by refrigeration.[75] Sweet corn in the husk retains its vitamin C well for two or three days, after which time a slow loss occurs.[68] Green peppers remain rich sources of ascorbic acid for several weeks if they do not become bruised or wilted.[15] Loss in apples after three months at 45 F. approximates 15 per cent, at six months 25 per cent and at one year 50 per cent.[76] Lemons and oranges stored at 45 or 50 F. for ten months show losses of from 10 to 30 per cent; no detectable loss occurs in two months.[15] Storage of ripe tomatoes at ordinary temperature for a month causes little change in the vitamin C content provided the fruit remains firm and free from bruises and rot.[77] Potatoes very slowly lose their vitamin C under market conditions; about a 50 per cent loss occurs in one year.[22] With some varieties, little loss is observed after from three to six months' storage.[78] Vitamin C in rutabagas, carrots, turnips and sweet potatoes is slowly lost over a period of several months.[68]

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[68] Fellers, C. R.: The Effect of Processing on Vitamins in Fruit and Vegetables: A Review, Bull. 338, Massachusetts State College Agricultural Experment Station, Amherst, December 1936.

[69] Bessey and King.[22]

[70] Nelson, E. M., and Mottern, H. H.: Effect of Lead Arsenate Spray on the Composition and Vitamin Content of Oranges, Am. J. Pub. Health **22**: 587 (June) 1932.

[71] Dunker, C. F.; Fellers, C. R., and Fitzgerald, G. A.: Stability of Vitamin C in Sweet Corn, Food Research **2**: 41 (Jan.) 1937.

[72] Mack, G. L.; Tressler, D. K., and King, C. G.: Vitamin C Content of Vegetables: II. Peas, Food Research **1**: 231 (May) 1936.

[73] Chace, E. M.: Health Problems Connected with Ethylene Treatment of Fruit, Am. J. Pub. Health **24**: 1152 (Nov.) 1934.

These facts demonstrate the great variation in "storage stability" of vitamin C and indicate the advantage of cold storage as compared with ordinary storage.

Freezing.—Preservation of fruits and vegetables by quick freezing has become an important commercial method.[79] Studies on the stability of vitamin C during freezing and storage in general have shown slight destruction, but often incidental processing operations such as shelling, washing and blanching lead to significant losses. Asparagus, apples, potatoes, strawberries, blackberries and orange juice are unaffected by freezing, while some losses are reported in preparatory operation with peas, beans and spinach. Freezing results in a certain amount of cellular disorganization, with liberation of enzymes from some types of plant tissues. Unless these products are properly blanched (to destroy enzymes) before freezing, considerable losses may occur during storage and thawing. Slow-thawing vegetables rapidly lost their vitamin C. Losses of from 80 to 90 per cent may occur in beans, peas or spinach in a few hours. Frozen foods should not be defrosted until ready to cook or serve raw. Losses are reduced to a minimum by cooking without preliminary defrosting.

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[74] Tressler, Mack and King, footnotes 63 and 75.

[75] Tressler, D. K.; Mack, G. L., and King, C. G.: Factors Influencing the Vitamin C Content of Vegetables, Am. J. Pub. Health **26**: 905 (Sept.) 1936.

[76] Batchelder, Esther L.: Vitamin C in Delicious Apples Before and After Storage, J. Nutrition **7**: 647 (June) 1934.

[77] Maclinn, W. A.; Fellers, C. R., and Buck, R. E.: Tomato Variety and Strain Differences in Ascorbic Acid (Vitamin C) Contents, Am. Soc. Horticultural Sc. **34**: 543, 1937.

[78] Fellers.[68] Wood.[89]

[79] Nelson, E. M., and Mottern, H. H.: Vitamin C Content of Frozen Orange Juice, Indust. & Engin. Chem. **25**: 216 (Feb.) 1933. Daniel and Munsell.[4] Fellers.[68] Tressler, Mack and King.[75]

Processing.—For several reasons, effort should be made to conserve the vitamin C values of the general food supply rather than to depend on the use of foods of special antiscorbutic properties. A larger percentage of the people than is generally realized, because of economic conditions, lack of knowledge or poor food habits, do not include citrus fruits, tomatoes or other richly antiscorbutic foods in their diet. These people necessarily depend on the general food supply for their source of this essential. Also the loss of important food qualities, such as flavor and the content of other vitamins, usually parallels the destruction of vitamin C In fact, the preservation of ascorbic acid may well be used as a general index to the care with which certain foods have been processed.

The preservation of vitamin C between harvesting and consumption is essentially a problem of combating oxidation, a process dependent on time and the kind and state of the tissue and influenced by temperature, access to air and presence of metals and enzymes. The destruction of vitamin C during the heating of tomato juice can be reduced two thirds by rendering the conditions anaerobic.[80] Spinach and cabbage cooked by methods excluding air are from 50 to 100 per cent richer in vitamin C than when prepared by open kettle cooking.[81] The water in which vegetables are cooked may contain sufficient oxygen to become an important factor of

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80. Kohlman, Eddy and others.[82] Sherman.[83]
81. Sherman and Smith.[3] Bessey.[15]

destruction.[82] This fact has led to the practice of starting vegetables in hot water.

Crushing or bruising of many vegetables such as spinach, cabbage, turnips and rutabagas causes the liberation of enzymes (oxidases) which in the presence of air catalyze the oxidation of ascorbic acid.[26] This is a rapid reaction and may lead to complete inactivation of the injured tissue in a few minutes. Starting the cooking in hot water quickly inactivates these enzymes. Shredded carrots, cabbage and other roots and leafy tissues rapidly decrease in vitamin C content and should therefore be prepared fresh before serving.

Vitamin C is more stable in an acid medium than in a neutral or slightly alkaline medium.[83] The addition of soda to cooking vegetables may produce a better color but will lead to increased loss of vitamin C. The vitamin C in tomato juice is naturally stable but becomes rapidly inactivated when neutralized or made alkaline with soda. In general, vitamin C is less subject to oxidation in those fruits and vegetables which are acid.

There is perhaps no single factor which accelerates the destruction of vitamin C so much as copper salts. The presence of air and the merest traces of copper lead to complete inactivation often in a few minutes. Contact with bare copper or utensils made of metal containing copper must be avoided. Quantities of copper so small as to be undetectable by the usual chemical methods will lead to immediate and complete destruction of the ascorbic acid in raw milk or cooking vegetables.[84] The insidious nature of this factor makes it very difficult to control, for, in addition to contamination by contact and from the water supply, many foods naturally contain enough copper to be active.

Drying [85] in itself is not detrimental to vitamin C, but for most foods it is almost impossible to prevent oxidation due to other factors sufficiently to prepare dehydrated products of important and lasting antiscorbutic values. Dehydrated foods have repeatedly failed in this respect when used in times of war. Many experiments have been reported showing that certain of the more acid fruits may be prepared so as to conserve a reasonable degree of their vitamin C activity, but commercial products cannot as yet be considered dependable in this respect.

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[82] Kohman, E. F.; Eddy, W. H., and Gurin, C. Z.: Vitamins in Canned Foods: XI. A Canned Food Diet, Indust. & Engin. Chem. **23:** 1064 (Sept.) 1931; Canning Tomato Juice without Vitamin C Loss, ibid. **25:** 682 (June) 1933.

[83] Sherman, H. C.: Chemistry of Food and Nutrition, ed. 5, New York, Macmillan Company, 1937.

[84] Whitnah, C. H.; Riddell, W. H., and Caulfield, W. J.: The Influence of Storage, Pasteurization, and Contamination with Metals on the Stability of Vitamin C in Milk, J. Dairy Sc. **19:** 373, 1936. Bessey.[15]

[85] Fellers.[63] Sherman.[83]

Pickling, salting, curing or fermenting usually results in complete loss of vitamin C. Fresh cucumbers contain from 5 to 8 mg. per hundred grams; salt pickles contain none. Fermented cider and other fruit juices are free from vitamin C. Sauerkraut and sauerkraut juice are unreliable antiscorbutic foods.[86] Commercial orange drinks which do not contain freshly prepared orange juice are practically free from ascorbic acid.[87] Vegetable juices rapidly lose their vitamin C because of active enzymes and a high p_H and therefore are usually poor antiscorbutic foods.

It is difficult to make general statements concerning the loss of vitamin C during such a variable process as cooking[88] or canning. Recent work is in essential agreement with the previous emphasis on the unfavorable "influence" of contact with copper utensils, access to air, prolonged heating and alkalinity. Modern commercial canning, with careful plant control, can prepare products with a minimum loss of vitamin C which are comparable to carefully cooked fresh food. However, no rule can cover all cases, for there are badly controlled canneries as well as bad cooks. The variations in processing are so great that the products from each process and perhaps each batch would have to be tested before one could be sure of the vitamin C value. Boiling of cabbage in an open kettle or cooking in a pressure cooker leads to almost complete destruction, while starting the cabbage in boiling water and testing both the cooking water and the cabbage produce about 25 per cent destruction and 25 per cent loss in the water.[64] Similar studies on spinach, turnip greens and green peas and beans lead to approximately the same conclusions. Commercial canning destroys from 50 to 85 per cent of the vitamin C in peas, lima beans, spinach and asparagus. Home-canned tomato juice is as satisfactory as commercial canned juice, and both are good antiscorbutic substances.[2] Vitamin C is stabilized by acid and therefore

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86. Sherman and Smith.[2] Fellers.[68]
87. Bessey.[15] Fellers.[68]
88. Sherman and Smith.[2] Gould, Tressler and King.[64] Fellers.[6] Sherman.[83]

The Vitamin C Content of Various Foods per Hundred Grams *

Food	Mg. of Ascorbic Acid	International Units
Apples		
Delicious	2.5	50
Jonathans	2.5	50
McIntosh	2	40
Winesaps	5	100
Golden delicious	4	80
Yellow Newton	5	100
Dried	0	0
Juice	See type of apple	
Apricots, fresh	1	20
Apricots, dried	8	160
Asparagus	40	800
Bananas	8	160
Beans		
Green	10	200
Green canned	4	80
Sprouted	10	200
Dried	0	0
Beef muscle, cooked	Trace	Trace
Beer	0	0
Beets	5	100
Beet leaves	35	700
Blackberries, fresh	3	60
Blackberries, frozen	3	60
Blueberries, low bush	4	80
Blueberries, high bush	10	200
Broccoli	50	1,000
Brussels sprouts	50	1,000
Butter	0	0
Cabbage, young, green	40	800
Cabbage, old	20	400
Cantaloup	40	800
Carrots	3	60
Cauliflower	30	600
Celery stalk	5	100
Chicory	10	200
Chard, Swiss, stalks	5	100
Chard, Swiss, leaves	20	400
Cheese, all varieties	0	0
Cherries, sweet	8	160
Cider, fresh	See apples	
Chicken meat, cooked	Trace	
Cod liver oil	0	0
Collard	60	1,200
Corn		
Sweet	10	200
Canned	6	120
Dried	0	0
Cranberries	10	200
Cucumbers	2	40
Currants, black	100	2,000
Currants, red	15	300
Dandelion greens	40	800
Dates, cured	0	0
Egg plant	5	100
Eggs	0	0
Endive	10	200
Escarole	7	140

*The Vitamin C Content of Various Foods per Hundred Grams *—Continued*

Food	Mg. of Ascorbic Acid	International Units
Figs, fresh	2	40
Figs, dried	0	0
Fish, fresh cooked	Trace	Trace
Fruit juice	See fruit	
Gooseberries	25	500
Grain, dried, all varieties	0	0
Grass, fresh, green	60	1,200
Grape juice	Trace	Trace
Grapefruit juice, fresh	40	800
Grapefruit juice, canned	30	600
Horseradish	100	2,000
Jelly	0	0
Kale	50	1,000
Kohlrabi	70	1,400
Leek	15	300
Lemon juice		
Fresh	60	1,200
Cold storage	40	800
Canned	50	1,000
Lettuce, green leaf	10	200
Lettuce, head	5	100
Lime juice, fresh	30	600
Liver, beef, cooked	10	200
Malt	0	0
Milk		
Human	6	120
Cow's, raw, fresh	2	40
Cow's, pasteurized	0 to 1	0 to 20
Cow's, dried	5	100
Mustard leaves	60	1,200
Nuts, all kinds	0	0
Okra	10	200
Onion	10	200
Orange juice, fresh	50	1,000
Orange juice, canned	45	900
Orange syrup	0	0
Papaya	40	800
Parsley	175	3,500
Parsnips	5	100
Peas, green	15	300
Peas, canned	5	100
Peaches, fresh	7	140
Peaches, dried	25	500
Pears, fresh	3	60
Pear juice, canned	Trace	Trace
Peppers, green	180	3,600
Peppers, red, ripe	230	4,600
Pineapple, fresh	25	500
Pineapple, canned	10	200
Plums	2	40
Potatoes, new	15	300
Potatoes, old	7	140
Prunes	2	40
Prunes, dried	0	0
Pumpkins	5	100
Quince	5	100
Radishes	12	240
Raisins	0	0

The Vitamin C Content of Various Foods per Hundred Grams—Continued*

Food	Mg. of Ascorbic Acid	International Units
Raspberries	15	300
Rhubarb	15	300
Rutabaga	30	600
Seeds, mature, dry (all varieties)	0	0
Spinach, fresh	60	1,200
Spinach, canned	5 to 10	100 to 200
Sauerkraut juice	0 to 5	0 to 100
Squash	3	60
Strawberries	50	1,000
Sweet potatoes	8	160
Swede	See rutabaga	
Tangerines	30	600
Tomatoes		
Green	20	400
Vine ripe	30	600
Artificial ripe	25	500
Tomato juice, fresh	30	600
Tomato juice, canned	25	500
Turnips	30	600
Turnip greens	60	1,200
Vegetable juices canned	0 to 5	0 to 100
Watercress	50	1,000
Watermelon	15	300
Yeast	0	0

* Unless otherwise indicated, these values are on fresh foods.

suffers less destruction during cooking in acid fruits and vegetables. Canned grapefruit and orange juice are about 70 to 90 per cent as potent as the fresh juice and, like most canned products, remain stable while canned but slowly lose their vitamin C when left open to the air.[87] Baked and boiled potatoes are reported to show only small losses unless overcooked.[89] Fruit jellies and jams are usually low in vitamin C. Warmed over foods are practically free from ascorbic acid. Canned or cooked apricots, peaches, plums and cherries are practically free from vitamin C. Freshly prepared apple sauce or apple pies retain from 20 to 30 per cent of the original vitamin C value of the apple.[68]

The use of canned sieved vegetables and fruit for children has increased at a rapid rate in the last few years.[90] In spite of the most careful methods of proc-

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89. Wood, E.: The Vitamin C Content of the Russel Burbank Potato of Idaho, Bull. 219, University of Idaho Agricultural Experiment Station, June 1935. Fellers.[68]

90. Fellers, C. R.: Vitamin Content of Important Foods in the Child's Diet, Am. J. Pub. Health **25**:1340 (Dec.) 1935. Hanning, Flora:

essing, partial destruction of vitamin C is unavoidable. A great deal of variation in this occurs, depending on the type of food, freshness and method of processing, but in general it has been shown that the loss of vitamin C is slightly greater for purées than for the corresponding canned whole vegetables. As a rule these products cannot be classed as potent antiscorbutic foods, although they may contribute materially to the diet because of the quantity consumed.

Milk is the most nearly perfect and the least dispensable food. Nevertheless, the vitamin C content of commercial milk, raw or pasteurized, is not dependable and should never be relied on to supply the requirements. The use of the titration method has led to a better understanding of those factors which may influence the vitamin C content of milk, such as aging, pasteurization and handling. Judging from a large number of determinations, both biologic and chemical, one may expect from 2 to 2.5 mg. per hundred cubic centimeters of ascorbic acid in fresh raw milk.[91] Even when the milk is cold this value slowly decreases, until a loss of from 20 to 30 per cent may occur in twenty-four hours. Sunlight or traces of copper accelerate this change.[92] Pasteurization by the widely used holding method, unless unusual precautions are observed, results in a loss of from 30 to 60 per cent. However, pasteurization by the short time high temperature process with absence of copper leads to slight inactivation.[93] Loss of vitamin C activity due to pasteurization, aging, reheating and diluting, such as usually occurs in preparing a formula for babies, results in a formula of very low antiscorbutic value.[15]

Although Hess[94] found that a pint of milk a day prevented infantile scurvy, the commercial milk of today, which contains from 0.3 to 1 mg. per hundred cubic centimeters, is a rather poor source of vitamin C. The 5 mg. of ascorbic acid represented by a pint of average commercial milk is far below that recommended for optimum nutrition. This fact has led to the general use of orange juice, tomato juice or potent antiscorbutic substances as a supplement to the infant diet. A breast-fed infant of a normally nourished mother receives from 20 to 40 mg. of ascorbic acid daily from the day of birth, because human milk is from four to six times richer in vitamin C than cow's or goat's milk.[95] The implication is strong that vitamin C should be a constituent of the infant's diet at a very early age.

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[91] Rasmussen, Russel, and Guerrant, N. B.: The Effect of Breed Characteristics and Stage of Lactation on the Vitamin C (Ascorbic Acid) Content of Cow's Milk, J. Nutrition **11**: 425 (May) 1936. Riddell, W. H.; Whitnah, C. H.; Hughes, J. S., and Leinhardt, H. F.: Influence of the Ration on the Vitamin C Content of Milk, J. Nutrition **11**: 47 (Jan.) 1936. King and Waugh.[93]

[92] Kon, S. K., and Watson, M. B.: The Effect of Light on the Vitamin C of Milk, Biochem. J. **30**: 2273 (Dec.) 1936. King.[23]

[93] King, C. G., and Waugh, W. A.: Effect of Pasteurization on the Vitamin C Content of Milk, J. Dairy Sc. **17**: 489, 1934. Schwartze, E. W.; Murphy, F. J., and Hann, R. M.: Studies on the Destruction of Vitamin C in the Boiling of Milk, J. Nutrition **2**: 325 (March) 1930.

[94] Hess, cited by Sherman.[83]

The ascorbic acid value of canned or dried milk[2] depends primarily on the method by which the milk is prepared or handled. Commercially evaporated milk which has been sterilized at a high temperature is almost free from vitamin C, while sweetened condensed milk which has been evaporated in vacuum at lower temperatures retains a proportion of vitamin C comparable to that of pasteurized milk. Several investigators have reported good conservation in dried milk.[68] However, most commercial preparations are practically free from vitamin C.[15]

The vitamin C concentrations of individual foods are summarized in the accompanying table. Most of the analyses reported were on fresh material. It is difficult to estimate values for cooked or processed food because of the lack of uniformity of methods and treatment. Lower vitamin C values than those given must be expected because of immaturity, lack of freshness, poor preservation, long storage or results of processing. The figures given are estimates derived after consideration of the available data,[96] including the results of many unpublished analyses from my own laboratory, and represent my judgment of the expected value of the raw edible portions under good market conditions. The value when the food is ready for serving will depend on the method of preparation. A recent United States Department of Agriculture bulletin[4] contains fuller details of the vitamin C contents of foods and also references to the literature.

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[95] Selleg, Iva, and King, C. G.: The Vitamin C Content of Human Milk and Its Variation with Diet, J. Nutrition **11**: 599 (June) 1936.

[96] Daniel and Munsell.[4] Fellers.[68] Lack of space prevents reference to many of the papers considered.

CHAPTER XXI

HUMAN REQUIREMENTS OF VITAMIN C

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It was early found that about twice as much vitamin C is required to prevent the first appearance of microscopic alterations in the teeth as to prevent the outward symptoms of scurvy in guinea pigs. It was also observed that in infants and children receiving enough vitamin C for protection against scurvy there may still develop a condition of latent or subacute scurvy accompanied by more or less severe injury to the teeth. It then became apparent that considerably larger quantities of vitamin C are required for good nutrition than for the prevention of scurvy.[1]

The first attempt at using human beings as subjects for the determination of requirements of vitamin C was made in Sweden in 1931 by Göthlin,[2] who measured the capillary resistance or fragility of two forcibly fed schizophrenic subjects during a period on a liquid diet which at first was devoid of vitamin C and later supplemented with increasing quantities of orange juice until the lowered capillary resistance was barely restored to normal. This point was reached on daily intakes of 0.7 and 1 cc. per kilogram of body weight respectively. Translated into modern terms, this would correspond to from 21 to 30 mg. of ascorbic acid for an adult weighing 60 Kg., or about 132 pounds. In Göthlin's opinion the disturbance in the strength of the capillaries as measured by his blood pressure method corresponds to the microscopic alterations in the teeth of guinea pigs as measured by the Höjer[3] tooth structure method, and by relating these two methods he concluded that a man weighing 60 Kg. requires from fourteen to twenty times as much vitamin C as a guinea pig weighing 300 Gm. for corresponding protection. When ascorbic acid became available Göthlin [4] used it in place of orange juice in this indirect method of determining human requirements with resulting values of from 19 to 27 mg. daily—quantities surprisingly close to the value of from 21 to 30 mg. calculated from the direct experiments on human subjects.

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[1] Sherman, H. C., and Smith, Sybil L.: The Vitamins, ed. 2, New York, Chemical Catalog Company, 1931, chapt. IV.

[2] Göthlin, G. F.: A Method of Establishing the Vitamin C Standard and Requirement of Physically Healthy Individuals by Testing the Strength of Their Capillaries, Skandinav. Arch. f. Physiol. **61**: 225 (May) 1931.

[3] Höjer, J. A.: Method for Determining the Antiscorbutic Value of a Foodstuff by Means of Histological Examination of the Teeth of Young Guinea Pigs, Brit. J. Exper. Path. **7**: 356 (Dec.) 1926.

This indirect method, however, has received less attention since knowledge of the chemical identity of ascorbic acid has made possible more or less satisfactory tests for its identification, and its availability in quantity has led to its use in metabolic studies with determinations in the urine and blood subsequent to the ingestion or injection of known quantities of the vitamin. Following the now classic studies of Harris and his associates,[5] Johnson and Zilva [6] and Hess and Benjamin [7] on the relation of urinary excretion to the intake of vitamin C as determined by indophenol titration, and of King and his associates,[8] Gabbe [9] and Farmer and Abt [10] on the significance of the concentration of ascorbic acid in blood as determined by the same indicator with certain modifications in technic, the outlook seemed encouraging toward the close of 1934 for the establishment of definite standards for judging vitamin C nutrition and requirements for various age groups. As is often the case, however, the problem has become more rather than less complicated with increased study. This is due chiefly to the fact that thus far no entirely specific method has been developed for the chemical determination of ascorbic acid. Among the numerous reports that have been published during the past three years there have been quite as many dealing with difficulties in methodology as with definite requirements. Brief mention of the more important sources of error in the determination of ascorbic acid in blood and urine has been given in a previous paper of the series.[11] Space does not permit the detailed discussion of methodology, which is almost necessary for the evaluation of data on which present recommendations of requirements are based. The problem is still further complicated by the multiplicity of methods that have been used to interpret data on metabolism in terms of quantitative requirements as reviewed in the following section.

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[4] Göthlin, G. F.: Human Daily Requirements of Dietary Ascorbic Acid, Nature **134:** 569 (Oct. 13) 1934.

[5] Harris, L. J.; Ray, S. N., and Ward, A.: The Excretion of Vitamin C in Human Urine and Its Dependence on the Dietary Intake, Biochem. J. **27:** 2011, 1933.

[6] Johnson, S. W., and Zilva, S. S.: The Urinary Excretion of Ascorbic and Dehydroascorbic Acids in Man, Biochem. J. **28:** 1393, 1934.

[7] Hess, A. F., and Benjamin, Helen R.: Urinary Excretion of Vitamin C, Proc. Soc. Exper. Biol. & Med. **31:** 856 (April) 1934.

[8] Yavorsky, M.; Almaden, Philip, and King, C. G.: The Vitamin C Content of Human Tissue, J. Biol. Chem. **106:** 525 (Sept.) 1934.

[9] Gabbe, E.: Bestimmung von Vitamin C im Blutserum, Klin. Wchnschr. **13:** 1389 (Sept. 29) 1934.

[10] Farmer, C. J., and Abt, A. F.: Ascorbic Acid Content of Blood, Proc. Soc. Exper. Biol. & Med. **32:** 1625 (June) 1935.

METHODS OF DETERMINING THE REQUIREMENTS OF VITAMIN C

The adequacy of vitamin C in human nutrition is determined by one or more of three measurements: (1) the resistance or fragility of the blood capillaries, (2) the excretion of ascorbic acid in the urine and (3) the content of ascorbic acid in the fasting blood. To make use of these measurements for the estimation of requirements of vitamin C involves the acceptance of certain standards based on repeated observations on healthy normal persons.

Capillary Resistance or Fragility.—In this method the strength of the blood capillary system is measured on the upper arm by the positive pressure technic of Göthlin [12] or by the negative pressure technic of Dalldorf.[13] It was at first anticipated that this would prove to be a very practical method for survey work in estimating the vitamin C reserves of large groups of people, but this has not proved to be the case, in this country at least. The literature on the use of the method has been reviewed recently in THE JOURNAL.[14] Since then, papers dealing with the usefulness and limitations of the method have appeared from the laboratories of both Göthlin and Dalldorf.

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[11] Bessey, O. A.: Vitamin C: Methods of Assay and Dietary Sources, J. A. M. A. **111:** 1290 (Oct. 1) 1938.

[12] Göthlin, G. F.: Outline of a Method for the Determination of the Strength of the Skin Capillaries and the Indirect Estimation of the Individual Vitamin C Standard, J. Lab. & Clin. Med. **18:** 484 (Feb.) 1933.

[13] Dalldorf, Gilbert: A Sensitive Test for Subclinical Scurvy in Man, Am. J. Dis. Child. **46:** 794 (Oct.) 1933. Dalldorf, Gilbert, and Russell, Hollis: The Effect of Cevitamic Acid Injections on Capillary Resistance, J. A. M. A. **104:** 1701 (May 11) 1935.

[14] Youmans, J. B.: The Present Status of Vitamin Deficiencies in Practice, J. A. M. A. **108:** 15 (Jan. 2) 1937.

According to Göthlin,[15] the capillary fragility test serves as a measure of the physiologically indispensable minimum requirement of vitamin C, for it can be used to determine the quantity of the vitamin necessary to maintain the capillary system on the borderline between a normal and a weakened condition. The test is also useful in detecting a rather severe degree of vitamin C undernutrition. From simultaneous tests for capillary fragility and the determination of the ascorbic acid content of the fasting blood it has been shown that at this borderline of capillary strength the ascorbic acid content of the blood lies between 0.1 and 0.14 mg. per hundred cubic centimeters, values considerably below the generally accepted minimum normal value of about 0.6 mg. per hundred cubic centimeters. Consequently there is a degree of vitamin C deficiency within the range in ascorbic acid content of the blood of from 0.1 to 0.6 mg. which cannot be detected by the capillary fragility test. In Göthlin's opinion the reason the capillary fragility test is considered of little value as a means of detecting vitamin C undernutrition in certain countries, while in Sweden where his work has been done it has been found so satisfactory, is that in countries where there is an abundance of vitamin C-containing foods blood values seldom fall as low as 0.14 mg. per hundred cubic centimeters, and consequently there is no reaction with the capillary fragility test. In Sweden, however, during the long winter months the vitamin C content of the diet is so low that capillary fragility develops in thousands and the test is very useful as a means of detecting the most severe deficiencies.

A more clinical slant to the problem is given by Sloan from Dalldorf's hospital laboratory.[16] Applying the test with negative pressure technic to several normal subjects, two subjects with bleeding but no scorbutic symptoms and a fairly large group of patients with all degrees of scorbutic symptoms from simple bleeding of the gums to severe scurvy, he has concluded that the capillary resistance test in the majority of cases gives dependable information concerning the presence or absence of vitamin C depletion but does not indicate the degree of depletion and gives falsely negative results in the presence of severe anemia.

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[15] Göthlin, G. F.: When Is Capillary Fragility a Sign of Vitamin C Subnutrition in Man? Lancet **2**: 703 (Sept. 18) 1937.

[16] Sloan, R. A.: A Comparison of Methods for Detecting and Grading Subclinical Scurvy, J. Lab. & Clin. Med. **23**: 1015 (July) 1938.

Urinary Excretion of Ascorbic Acid.—The twenty-four hour output of ascorbic acid in the urine as determined by indophenol titration according to various modifications of the original Tillmans method[17] has been used more generally than any other value as a measure of vitamin C nutrition and excretion after test doses as a means of calculating requirements. In the early work of Harris and his associates[18] the term "resting level" of vitamin C was used to indicate the day-by-day excretion of the vitamin, with a value of about 10 mg. representing the borderline between deficiency and adequacy, 20 mg. a moderately low intake and 40 mg. a liberal intake of the vitamin. Somewhat later, on finding that adult subjects on a daily intake of 25 mg. of ascorbic acid, selected as representing the "minimum-optimal" requirement, excreted about 13 mg. daily, the Harris group[19] concluded that if a subject excretes less than 13 mg. of ascorbic acid a day and fails to respond by a marked increase in excretion on the first or second day to a test dose of 700 mg. per 10 stone (140 pounds, 63.5 Kg.) of body weight, his diet has contained less than the reputed minimum-optimal quantity of vitamin C. This excretion value of 13 mg. a day is often used without further qualification as a standard of adequacy, but, since it represents an intake within the range of the physiologic minimum requirement established by Göthlin,[2] as noted earlier, it should be considered as indicating the borderline of adequacy rather than an optimum condition. According to van Eekelen,[20] a daily excretion of 40 mg. indicates that the subject is in a state of vitamin C saturation.

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17. Tillmans, J.; Hirsch, P., and Hirsch, W.: Das Reduktionsvermögen pflanzlicher Lebensmittel und seine Beziehung zum Vitamin C: I. Der reduzierende Stoff des Citronensaftes, Ztschr. f. Untersuch. d. Lebensmitt. **63**: 1 (Jan.) 1932. Harris, Ray and Ward.[5] Bessey, O. A., and King, C. G.: The Distribution of Vitamin C in Plant and Animal Tissues and Its Determination, J. Biol. Chem. **103**: 687 (Dec.) 1933.

18. Abbasy, M. A.; Harris, L. J.; Ray, S. N., and Marrack, J. R.: Diagnosis of Vitamin C Subnutrition by Urine Analysis: Quantitative Data, Lancet **2**: 1399 (Dec. 21) 1935.

19. Harris, L. J.; Abbasy, M. A., and Yudkin, J.: Vitamins in Human Nutrition: Vitamin-C Reserves of Subjects of the Voluntary Hospital Class, Lancet **1**: 1488 (June 27) 1936.

20. van Eekelen, Marie; Emmerie, Adrianus, and Wolff, L. K.: Ueber die Diagnostik der Hypovitaminosen A und C durch die Bestimmung dieser Vitamine im Blut, Ztschr. f. Vitaminforsch. **6**: 150 (April) 1937.

It has been recognized from the first that a better picture of vitamin C nutrition can be obtained by the so-called test dose or saturation method than by a determination of the resting level of excretion alone. Briefly, this method consists in determining the daily excretion during a period in which large doses of ascorbic acid are given to saturate the tissues. In the use of the method there has been little agreement as to general procedure and interpretation. Opinions differ as to the size of the test dose, the method of administration, the end point of saturation and the final calculation of the data.

A dosage of 700 mg. per 10 stone (140 pounds) of body weight, as first recommended by Harris and his associates,[19] has been used most frequently, although doses as low as 100 mg. and as high as 1,000 mg. have been reported. When the ascorbic acid is taken by mouth, as is commonly the case, the excretion of 50 per cent or more of the entire test dose within twenty-four hours, or of the half-day dose within twelve hours,[21] is commonly used as the criterion of saturation, although this will depend on the size of the test dose, for the larger the dose the lower the percentage excreted. This makes it difficult to compare observations of different investigators who have used test doses varying in size.

It has been shown that the peak of excretion of ascorbic acid in the urine occurs within from four to six hours following oral administration,[22] and within from one to three hours following intravenous administration.[23] Subcutaneous injection is preferred to intravenous by van Eekelen and Heinemann,[22] who consider that when the test dose is given intravenously the concentration in the blood is raised so suddenly that a transitory overflow into the urine results before the tissues are saturated. In their experience a test dose not exceeding 300 mg. when given subcutaneously to a saturated subject, is entirely excreted within six hours, with the peak occurring just before the end of the third hour. According to Baumann,[24] the best insight into the behavior of saturation is obtained by the oral administration over several days of small doses of ascorbic acid (50 mg. for small children, and 100 mg. for older children and adults). Under such conditions healthy persons excrete from 60 to 80 per cent of the test dose on the third or fourth day. He has noted a wide range in the saturation capacity of different persons as thus determined—the span ranging from 0 to 40 mg. of ascorbic acid per kilogram of body weight.

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[21] Jezler, A., and Kapp, H.: Zur Frage des Vitamin C-Defizits, Ztschr. f. klin. Med. **130:** 178 (June 10) 1936.

[22] Hawley, Estelle E., and Stephens, D. J.. Rate of Urinary Excretion of Test Doses of Ascorbic Acid, Proc. Soc. Exper. Biol. & Med. **34:** 854 (June) 1936. van Eekelen, Marie, and Heinemann, M.: Critical Remarks on the Determination of Urinary Excretion of Ascorbic Acid, J. Clin. Investigation **17:** 293 (May) 1938.

[23] Hawley and Stephens.[22] Ralli, Elaine P.; Friedman, G. J., and Kaslow, M.: An Excretory Test for Vitamin C Deficiency and Subnutrition, Proc. Soc. Exper. Biol. & Med. **36:** 52 (Feb.) 1937. Wright, I. S.; Lillenfeld, Alfred, and MacLenathen, Elizabeth: Determination of Vitamin C Saturation: A Five Hour Test After an Intravenous Test Dose, Arch. Int. Med. **60:** 264 (Aug.) 1937. Faulkner, J. M., and Taylor, F. H. L.: Observations on the Renal Threshold for Ascorbic Acid in Urine, J. Clin. Investigation **17:** 69 (Jan.) 1938.

A difference has been shown recently in the behavior of various persons to continued administration of ascorbic acid after the saturation point has been reached, as shown by a sudden and marked increase in excretion of ascorbic acid. Instead of additional increases with continued high doses, as might be expected if a real state of saturation had been reached, Hamel [25] found in some instances no further rise for several days. This plateau of excretion, indicating a disappearance of ascorbic acid after the body reservoirs had been filled, he attributed to a form of specific dynamic action, which should be taken into consideration in estimating requirements by the test dose method.

As a qualitative survey method for determining the state of vitamin C nutrition of large groups of subjects, the test dose method in its simplest form is undoubtedly of some value, as will be shown later. Advantage has been taken of the time interval before the peak of excretion has been reached after oral administration of the test dose to shorten the period of collection of urine in routine surveys of school children.[26] In the application of the method to the determination of the extent of vitamin C undernutrition of any age group, various procedures have been followed. The total quantity of ascorbic acid required before saturation is reached is sometimes referred to as the "saturation deficit," and the extent of this deficit is considered an index of the nutritional status of the subject with respect to vitamin C.[27] The number of days required to reach saturation on a given test dose serves as another index.[21] If a prolonged period on a vitamin C free diet precedes the saturation tests, the difference between intake and excretion up to the point of saturation is considered to be a measure of the maximum tissue reserves of the subject.[28]

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[24] Baumann, T.: Die Bestimmung der physiologischen Spanne der C-Vitamin Sättigung des Organismus, Klin. Wchnschr. **16:** 1246 (Sept. 4) 1937.

[25] Hamel, P.: Ueber die Vitamin C-Bilanz des Menschen: II. Belastungsversuche zur Bestimmung des täglichen Verbrauches und des Sättigungsdefizits, Klin. Wchnschr. **16:** 1105 (Aug. 7) 1937.

[26] Harris, L. J., and Abbasy, M. A.: Nutrition Surveys: A Simplified Procedure for the Vitamin-C Urine Test, Lancet **2:** 1429 (Dec. 18) 1937.

Of the various procedures that have been developed for the application of the test dose method to the determination of actual requirements of vitamin C, two or three will be described in some detail as representative of methods designed to determine vitamin C requirements ranging from minimum to maximum or representing barely adequate to presumably optimal nutrition. At the lower end of the scale may be considered a method developed by Widenbauer [29] and used in an extensive investigation of human requirements. In a preliminary period of several days, during which the subject is kept on a uniform diet containing very little vitamin C, the titration value of the urine is determined by the Harris and Ray method [5] on two twelve hour samples preserved with 10 per cent glacial acetic acid, and the average daily value thus obtained is considered as a blank to be deducted from the final average excretion. Test doses of from 200 to 500 mg. of ascorbic acid per day are then administered orally until saturation is reached, as shown by an excretion of at least 50 per cent of the half-day dose in twelve hours. Then follows a period of adjustment of the daily dose of ascorbic acid to the quantity required to give a titration value slightly higher than the preliminary blank value. When this dosage has been established, the same quantity is given over a final period of seven days with daily titrations of the urine to determine the average daily excretion value. The final calculation of requirement is made by subtracting from the average daily intake during the final period the average daily excretion in the same period minus the average daily excretion of the preliminary period. As the values thus obtained represent a bare equilibrium between intake and output, they may be considered as minimum requirements providing for no margin of safety.

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[27] Gander, J., and Niederberger, W.: Ueber den Vitamin C-Bedarf alter Leute, München. med. Wchnschr. **83:** 1387 (Aug. 21) 1936.

[28] O'Hara, Patricia H., and Hauck, Hazel M.: Storage of Vitamin C by Normal Adults Following a Period of Low Intake, J. Nutrition **12:** 413 (Oct. 10) 1936.

[29] Widenbauer, F.: Der Vitamin C Haushalt des Menschen unter verschiedenen Verhältnissen, Klin. Wchnschr. **16:** 600 (April 24) 1937.

The scheme followed by van Eekelen and his associates[30] consists essentially in first saturating the subject on a daily dose of 250 mg. of ascorbic acid, then depleting the reserves by a period of several weeks on a vitamin C free diet, and finally saturating again as in the first period, the end point in both being a sudden and marked increase in excretion. The total intake of ascorbic acid in the second saturation period divided by the number of days before saturation is considered as the daily use or actual requirement. Since the final period is one of saturation rather than of bare equilibrium, the values obtained represent more liberal allowances than those of Widenbauer. The method has been criticized, however,[31] as giving entirely different results, depending on the length of the intermediate depletion period, a point which will be discussed later.

A somewhat different procedure for determining maximum or optimal requirements but also depending on saturation tests has been developed by Hauck.[32] Briefly, this consists in first saturating the tissues by daily intakes of 200 mg. of ascorbic acid for six days—a period found long enough with the subjects studied to insure complete saturation as determined by the increase in excretion following a single test dose of 400 mg. The entire test is repeated two or three times to establish the individual response to the 400 mg. dose when saturation is complete. Finally a series of similar tests is run on graded doses of ascorbic acid until the least quantity is found which will induce a similar response to the test dose of 400 mg. as obtained in the preliminary tests. With the provision made in this method for complete saturation of the tissues and for individual standards of comparison, the values obtained may be considered to represent maximum requirements.

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[30] van Eekelen, Marie: On the Metabolism of Ascorbic Acid (Vitamin C), Acta brev. Neerland. **5**: 165 (Dec. 14) 1935. van Eekelen, Marie; Heinemann, M., and van Wersch, H. J.: On the Daily Requirements for Ascorbic Acid of Man, ibid. **6**: 107 (June 20) 1936. van Eekelen, Marie: On the Amount of Ascorbic Acid in Blood and Urine: The Daily Human Requirement for Ascorbic Acid, Biochem. J. **30**: 2291 (Dec.) 1936.

[31] Göthlin, G. F.; Frisell, E., and Rundquist, N.: Experimental Determinations of the Indispensable Requirements of Vitamin C (Ascorbic Acid) of the Physically Healthy Adult, Acta med. Scandinav. **92**: 1 (June 6) 1937.

[32] Hauck, Hazel M.: Unpublished data.

Concentration of Ascorbic Acid in the Blood.—The content of reduced ascorbic acid in fasting blood of normal subjects does not vary with age but within certain limits is dependent on previous dietary intake of vitamin C. In the data reported in the literature [33] there appears to be rather closer agreement as to the lower than the higher limits of normality, the range extending from a minimum of about 0.6 mg. to a maximum of from 1.5 to 2 mg. or more per hundred cubic centimeters.

Abt and Farmer [34] have stated that "the cevitamic acid (reduced) content of the blood varies directly with the vitamin C content of the previous diet." In the opinion of Greenberg, Rinehart and Phatak,[33e]

> "the estimation of the reduced plasma ascorbic acid is only a measure of the immediate nutritive or metabolic level relative to vitamin C, and is dependent on recent dietary habits to a large degree. Although it is an index of the vitamin C nutrition at the time of the test, in a single case a low level does not imply tissue injury or scurvy (either clinical or subclinical). The latter results from the operation of suboptimal or low metabolic levels over some period of time. Conversely, a good or high level would not indicate that deficiency had not operated to produce tissue injury in the past. A more accurate index of the degree of deficiency existing at the time in any given case can be had by means of serial determinations following administration of known vitamin C supplements."

Portnoy and Wilkinson [35] have observed occasional subnormal plasma values in subjects showing no other test

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33. (a) Abt, A. F.; Farmer, C. J., and Epstein, I. M.: Normal Cevitamic (Ascorbic) Acid Determinations in Blood Plasma and Their Relationship to Capillary Resistance, J. Pediat. **8:** 1 (Jan.) 1936. (*b*) Taylor, F. H. L.; Chase, D., and Faulkner, J. M.: The Estimation of Reduced Ascorbic Acid in Blood Serum and Plasma, Biochem. J. **30:** 1119 (July) 1936. (*c*) Deggeller, J. C.: Researches on the Vitamin C Content of the Blood. Acta brev. Neerland. **6:** 64 (July 4) 1936. (d) Stephens, D. J., and Hawley, Estelle E.: The Partition of Reduced Ascorbic Acid in Blood, J. Biol. Chem. **115:** 653 (Oct.) 1936. (*e*) Greenberg, L. D.; Rinehart, J. F., and Phatak, N. M.: Studies on Reduced Ascorbic Acid Content of the Blood Plasma, Proc. Soc. Exper. Biol. & Med. **35:** 135 (Oct.) 1936. (*f*) Ingalls, T. H.: Studies on the Urinary Excretion and Blood Concentration of Ascorbic Acid in Infantile Scurvy, J. Pediat. **10:** 577 (May) 1937. (*g*) Pijoan, M., and Eddy, E.: Ascorbic Acid Content of Red Cells and Plasma, J. Lab. & Clin. Med. **22:** 1227 (Sept.) 1937. (*h*) Baumann.[24] (*i*) Sloan.[16]

34. Abt. A. F., and Farmer, C. J.: Cevitamic Acid Content of the Blood Plasma, Am. J. Dis. Child. **54:** 682 (Sept.) 1937.

35. Portnoy, B., and Wilkinson, J. F.: Vitamin C Deficiency in Peptic Ulceration and Hematemesis, Brit. M. J. **1:** 554 (March 12) 1938.

for vitamin C deficiency and have suggested a similar explanation. In their opinion, however,

> the finding of a normal or high plasma ascorbic acid shows the patient to have good reserves of the vitamin (always assuming that a large dose of vitamin C-containing food has not been taken before the test begins) and a single estimation should be sufficient without corroborative evidence from other tests.

Relationship Between the Ascorbic Acid Content of Blood and Urine.—A straight line relationship between the initial ascorbic acid content of the blood and the total quantity of ascorbic acid required for saturation as determined by excretion in the urine was demonstrated by van Eekelen and his associates,[20] who showed that with an ascorbic acid content of the blood of about 0.4 mg. per hundred cubic centimeters a total of 2 Gm. of ascorbic acid was required before saturation was reached as compared with only 1 Gm. for an initial blood value of 0.8 mg. per hundred cubic centimeters. They consider that the degree of vitamin C saturation of a subject can be estimated simply by a single blood determination in place of the tedious urinary excretion tests, a blood content of from 0 to 0.4 mg. of ascorbic acid per hundred cubic centimeters denoting a poor, from 0.4 to 0.8 mg. a moderate, from 0.8 to 1.2 mg. a very good and above 1.2 mg. an excellent state of saturation. A similar relationship was noted by Baumann,[24] who found that with blood values as low as 0.45 mg. per hundred cubic centimeters from 20 to 40 mg. of ascorbic acid per kilogram of body weight was required for saturation, whereas a content of over 1.4 mg. per hundred cubic centimeters indicated a high degree of saturation.

Evidence leading to the conclusion that ascorbic acid has a definite renal threshold value at a blood concentration of about 1.4 mg. per hundred cubic centimeters has been reported by Faulkner and Taylor[23] with the conclusion that blood values at or above this level indicate saturation and below this level unsaturation in varying degree. Others believe that the threshold value varies with the person[36] and more particularly that there are two types of persons with high and low threshold values respectively.[37] This would account for the wide span which has been noted in the saturation capacity of different individuals.[38] Neuweiler's [37] evidence in support of this hypothesis is of interest also in indicating when it is safe to use blood values alone as a measure of saturation. Among the forty-three nonpregnant women whom he studied, all but one out of twelve with initial blood values ranging from 1 to 1.5 mg. per hundred cubic centimeters came within the normal four day limit of saturation, according to the Jezler and Kapp method,[21] while all five subjects with blood values under 0.6 mg. per hundred cubic centimeters required from six to eight days to complete the test.

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[36] Lund, H.: Eine quantitative und spezifische Methode zur Ascorbinsäuretitration im Ha n und zur Bestimmung des Schwellenwertes, Klin. Wchnschr. **16**: 1085 (July 31) 1937.

The remaining twenty-seven subjects with blood values decreasing from 0.95 to 0.6 mg. per hundred cubic centimeters gave inconsistent results, some completing the test within four days while others with the same initial blood values required a longer time. Neuweiler concluded that while both high and low blood values may furnish sufficient evidence of the vitamin C nutrition of a person, intermediate values of from about 0.6 to 1 mg. per hundred cubic centimeters should not be considered alone but in connection with the results of saturation tests.

Oral and intravenous tolerance tests involving simultaneous determinations of the blood and urine at frequent intervals following the administration of a single massive dose of ascorbic acid orally or intravenously have been recommended by Portnoy and Wilkinson [35] as furnishing the most reliable information concerning the state of vitamin C nutrition. Individual variations in the rate of absorption of ascorbic acid from the intestinal tract or possible destruction of the vitamin are considered to render the oral test somewhat less satisfactory than the intravenous tolerance test. It is suggested that in the latter method, when used as a routine, blood tests may be omitted and determinations made only of the ascorbic acid excretion during the first five hours following the injection. This abbreviated test, however, may not be valid in cases of damage to the kidneys, which is revealed by simultaneous tests on the blood and urine.[39]

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[37] Wachholder, K., and Hamel, P.: Ueber die Vitamin C-Bilanz des Menschen: III. Täglicher Bedarf und Güte des Versorgungszustandes

[38] Baumann.[24] Hamel.[25]

REQUIREMENTS OF VARIOUS AGE GROUPS

Infants.—The vitamin C reserves of the infant at birth depend on the state of nutrition of the mother, with umbilical cord blood usually giving higher values than the corresponding maternal venous blood,[40] possibly because of increased use of the vitamin by the mother during labor. In a group of twenty-two hospitalized cases, Braestrup [41] obtained average values of 0.26 mg. of ascorbic acid per hundred cubic centimeters for the maternal blood, 1.07 mg. for the cord blood and 0.69 mg. for capillary heel blood of the infants thirty minutes after birth. Ten days after birth these capillary blood values had dropped to an average of 0.27 mg. per hundred cubic centimeters. The mothers in this study had been on diets very deficient in vitamin C before admission to the hospital, which may account for the low value of the maternal blood.

The rapid decrease in ascorbic acid content of the blood during the first ten days, as noted, suggests that the reserves with which the infant is supplied at birth are sufficient for only a few days and that thereafter vitamin C must be supplied either through breast milk or as a supplement to artificial feeding in order to maintain the blood concentration at the level which seems indicative of a good vitamin C reserve at any age. Mindlin [42] reports an average plasma ascorbic acid value of 1 mg. per hundred cubic centimeters for a group of about twenty infants at the age of 2 weeks, as compared with 0.3 mg. for a comparable group artificially fed from birth or for at least six days before the test, and from 0.4

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[39] Wright, I. S., and MacLenathan, Elizabeth: Vitamin C Saturation—Kidney Retention After an Intravenous Test Dose of Ascorbic Acid, Proc. Soc. Exper. Biol. & Med. **38:** 55 (Feb.) 1938.

[40] Abt, Farmer and Epstein.[33a] Wahren, H., and Rundquist, O.: Ueber den Ascorbinsäuregehalt des Blutes von Mutter und Frucht, Klin. Wchnschr. **16:** 1498 (Oct. 23) 1937. Braestrup, P. W.: Studies of Latent Scurvy in Infants: 1. Capillary Resistance of Newly-Born Children and During the First Year of Life; 2. Content of Ascorbic (Cevitamic) Acid in the Blood-Serum of Women in Labour and in Children at Birth, Acta paediat. **19:** 320, 328, 1937. Nielsen, H. E.: Serumascorbinsyren under Normale og Forskellige Pathologiske Forhold, Bibliot. f. læger. **130:** 20 (Jan.) 1938.

[41] Braestrup, P. W.: Studies of Latent Scurvy in Infants: III. The Content of Reduced Ascorbic Acid in Blood Plasma in Infants, Especially at Birth and in the First Days of Life, J. Nutrition **16:** 363 (Oct.) 1938.

[42] Mindlin, R. L.: The Relation Between Plasma Ascorbic Acid Concentration and Diet in the Newborn Infant, J. Pediat. **13:** 309 (Sept.) 1938.

to 0.8 mg. for a smaller group of artificially fed infants receiving 20, 30 or 75 mg. of ascorbic acid for a week or more, the 20 mg. dose giving as good results as the larger amounts. Braestrup [41] also found that initially low blood levels could be raised to from 0.56 to 0.76 mg. per hundred cubic centimeters by a 20 mg. supplement of ascorbic acid daily but were not affected by 10 mg. These data, although fragmentary, suggest that to maintain the ascorbic acid content of the blood of artificially fed infants at low normal values during the first weeks of life not less than 20 mg. of ascorbic acid daily is needed. In Braestrup's subjects, whose weights were given, this allowance is equivalent to from 5 to 6 mg. per kilogram of body weight.

On the basis of estimates of the vitamin C intakes of breast-fed and artificially fed infants from 9 to 12 days of age excreting in twenty-four hours more than 50 per cent of test doses of 30 mg. of ascorbic acid per kilogram of body weight, injected subcutaneously, Neuweiler [43] also arrived at the conclusion that the vitamin C requirement of very young infants is in the vicinity of 6 mg. per kilogram of body weight and concluded that the diet of infants, especially the artificially fed, should be supplemented at this early age (less than 2 weeks) with fruit juices or a pure preparation of ascorbic acid.

Formerly it was considered unnecessary to supplement the diet of artificially fed infants with vitamin C before the age of 3 or 4 months but the most recent (December 1937) recommendation of the Technical Committee on Nutrition of the Health Organization of the League of Nations [44] for artificially fed infants is to add to the diet 10 to 30 cc. of orange juice, or tomato juice equivalent to at least from 5 to 15 mg. of ascorbic acid daily.

A condition of subacute vitamin C deficiency has been recognized [45] as occurring occasionally in infants as early as the second day of life. The condition is described as a vague nutritional upset with disturbance in water metabolism and excessive loss in weight

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43. Neuweiler, W.: Vitamin C—Stoffwechsel bei Neugeborene, Ztschr. f. Vitaminforsch. **6**: 75 (Jan.) 1937.

44. Report by Technical Commission on Nutrition on Work of the Third Session, Bull. Health Organ., League Nations **7**: 475, 1938.

45. Ley, L.: Die Bedeutung des Vitamin C für das Neugeborene, Klin. Wchnschr. **16**: 1425 (Oct. 9) 1937.

responding promptly to treatment with ascorbic acid. Ley conducted urinary excretion tests on four male infants 4 days of age, two of whom (one breast-fed and one artificially fed) showed these symptoms of vitamin C deficiency. Although the absolute values reported are probably of no great significance, it is of interest that the excretion of ascorbic acid of the two normal infants was about twice that of the other two. The artificially fed infant was then given 50 mg. and the mother of the breast-fed infant 300 mg. of ascorbic acid daily, following which both infants showed marked clinical improvement. The urine tested after ten or twelve days had increased in volume and ascorbic acid content, and the ascorbic acid content of the breast milk had increased from 3.57 to 6.07 mg. per hundred cubic centimeters. From these and other observations, Ley concluded that there is a wide variation in the vitamin C needs of individual infants during the first few weeks of life, owing to differences in functional capacity and the oxidation processes of intermediary metabolism.

From analyses by the customary indophenol titration method of tissues and organs of infants dying at birth or at varying intervals up to the age of 4 months, Ingalls[46] has shown progressive exhaustion of vitamin C reserves with increasing age. Even more convincing evidence of the effects of lack of vitamin C in this early period of life was shown by Ingalls in the case histories and histologic sections of the bones and costochondral junctions of a small group of premature infants receiving only pasteurized mother's milk and dying before the age of 4 months. These showed unmistakable signs of vitamin C deficiency not detected during life. Accordingly, Ingalls feels that some source of vitamin C should be given as early as the first week in life. "This is particularly important for premature infants, for the artificially-fed and for those suffering from disease."

Szent-Györgi[47] once made the statement "ein optimal funktionierender Körper kann nicht durch Vitamin zufuhr verbessert werden." Later in illustrating this principle he[48] showed that while a laboratory guinea pig may be kept in a presumably satisfactory and healthy condition and be protected from outward symptoms of vitamin C deficiency on from 1.5 to 2 mg. of ascorbic acid, it requires a much larger dose for protection against diphtheria and other bacterial toxins, and in its own natural environment may consume from 20 to 40 mg. of ascorbic acid daily from green food. This quantity also represents the dose required for complete saturation of the animal's tissues. In his opinion, in the long run the physiologic quantity of any vitamin will be found to be the amount which in its original environment the organism in question consumes daily. Applied to the vitamin C requirements of infants, this might be construed to mean the quantity of ascorbic acid furnished by the day's supply of breast milk of the best quality.

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[46] Ingalls, T. H.: Ascorbic Acid Requirements in Early Infancy, New England J. Med. **218**: 872 (May 26) 1938.

[47] Szent-Györgi, Albert: Die medizinische Bedeutung des Vitamins C, Deutsche med. Wchnschr. **60**: 556 (April 13) 1934.

Breast milk has long been considered much richer than cow's milk in vitamin C. Chemical tests have confirmed this belief but have shown fairly wide variations in content, depending largely on the food supply of the lactating woman. (Requirements for lactation will be discussed later.) However, the range in concentration and average values reported by investigators in different countries using similar methods of titration with 2,6 dichlorophenolindophenol are surprisingly uniform.[49] The values taken as a whole show that breast milk from women on satisfactory diets may be expected to furnish from 4 to 7 or 8 mg. per hundred cubic centimeters during the first few months of lactation. In fact, a concentration below 4 mg. per hundred cubic centimeters has been considered[50] to indicate a vitamin C deficiency in the tissues of the lactating woman.

Estimates of the daily consumption of vitamin C by breast-fed infants vary according to the values selected as most representative of the average concentration of the vitamin in breast milk. Selleg and King [49d] have estimated that under optimal conditions with a fluid intake of 21 ounces (630 cc.) an infant may receive as much as from 40 to 50 mg. of ascorbic acid daily from breast milk during the first few weeks. Widenbauer [51] has reported values ranging from 18 to 78 mg. in the twenty-four hour supply of breast milk from wet nurses in different stages of lactation and at the height of saturation by the test dose method. The highest values under these conditions are, naturally, not representative of the ordinary content of ascorbic acid in breast milk. Ingalls,[46] using 4 mg. per hundred cubic centimeters as an average value, has estimated that the normal infant during the first three months of life, in consuming from 500 to 1,000 cc. of breast milk, receives the equivalent of from 20 to 40 mg. of ascorbic acid daily. Braestrup [41] considers that with an average milk consumption of 150 cc. per kilogram of body weight a breast-fed infant may receive a minimum of 3 mg. and a maximum of 8 mg. of ascorbic acid per kilogram of body weight daily during the first few months of life.

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[48] Szent-Györgi, Albert: Les propriétés thérapeutiques des vitamines, Presse méd. **51:** 995 (June 25) 1938.

[49] (*a*) Neuweiler, W.: Ueber den Gehalt der Frauenmilch an Vitamin C, Ztschr. f. Vitaminforsch. **4:** 39 (Jan.) 1935. (*b*) Harris, L. J., and Diagnosis (

mit Vitamin C, Klin. Wchnschr. Iva, and King, C. G.: The Vitamin C Content of Human Milk and Its Variation with Diet, J. Nutrition **11:** 599 (June) 1936. (*e*) Widenbauer, F., and Kühner, A.: Ascorbinsäurestudien an stillenden Frauen, Ztschr. f. Vitaminforsch. **6:** 50 (Jan.) 1937.

[50] Baumann, T., and Rappolt, L.: Untersuchungen zum C-Vitaminstoffwechsel: II. Ueber die C-Vitaminausscheidung und den C-Vitaminbedarf laktierender Frauen, Ztschr. f. Vitaminforsch. **6:** 8 (Jan.) 1937.

None of these calculated intakes from breast milk of good quality fall below and most of them are considerably above the estimated requirements reviewed and far above the 1937 recommendation of the League of Nations Committee, as previously noted.

In spite of differences in the methods which have been used in estimating the vitamin C requirements of infants, the recommended allowances fall within the comparatively narrow range of from 5 to 8 mg. of ascorbic acid per kilogram of body weight or from 20 to 50 mg. daily after the first few days. These requirements may be met, during the first few months, by breast feeding alone under optimal conditions. However, it may be safer and more economical to supply some of the vitamin C directly to the infant rather than rely entirely on breast feeding. This practice is even more necessary for infants receiving pooled mother's milk instead of breast feeding. A 27 per cent loss of ascorbic acid was reported for samples of mother's milk held in a refrigerator for eighteen hours,[49d] and samples of pasteurized pooled mother's milk as deliv-

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51. Widenbauer, F.: Der Vitamin C-Haushalt stillender Mütter, Ernährung **2**: 64 (Feb.) 1937.

ered to a hospital from a directory for mother's milk were found to contain only 0.3 mg. of ascorbic acid per hundred cubic centimeters or less than one tenth as much as fresh breast milk of average quality.[46]

The ascorbic acid content of cow's milk ranges from 1 to about 2.6 mg. per hundred cubic centimeters for raw and from 0.4 to 1 mg. for pasteurized milk.[52] The probable extent of reduction of these initial low values by the time the milk is ready for consumption by the artificially fed infant has been shown by Hawley [53] in analyses for ascorbic acid of samples of milk as taken from various delivery wagons to a city health bureau laboratory for bacteriologic count.

The raw milk sampled had an average ascorbic acid content of 1.65 mg., the pasteurized milk 0.9 mg. and formulas prepared from the milk for use in the hospital nursery 0.61 mg. per hundred cubic centimeters.

According to Hawley, "if non-glass cooking utensils are used in the home, even further reduction is probable. As a result the average formula of the infant (600-750 cc.) would supply only 4 to 6 mg. of vitamin C and cannot, therefore, be considered an adequate source of this vitamin."

These figures for breast milk, pooled mother's milk, and cow's milk, as modified for infant feeding, afford rather convincing evidence of the advisability of furnishing supplementary sources of vitamin C to all infants at a very early age, the need increasing from breast-fed infants to those receiving milk mixtures.

The reluctance of some physicians to recommend vitamin C supplements at an earlier age and in more generous quantities than formerly may be due in part to hesitation to apply to vitamin C the doctrine of the newer knowledge of nutrition with its distinction between the terms "adequate" and "optimal." Possibly an infant protected against the earliest detectable sign of capillary fragility may be thought to be receiving adequate vitamin C. To the physician formerly accustomed to seeing many cases of unmistakable infantile scurvy, protection against scurvy may even indicate an adequate provision of the vitamin but in neither case can the state of nutrition be considered optimal if there are no reserves to meet unexpected demands—no margin of safety.

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[52] Daniel, Esther P., and Munsell, Hazel E.: Vitamin Content of Foods, U. S. Dept. Agr., miscellaneous publication 275, June 1937, p. 175.

[53] Hawley, Estelle E.: Vitamin C Content of Milks: Raw, Pasteurized and Baby Formulae, J. Am. Dietet. A. **14**: 275 (April) 1938.

Probably the best indication of whether or not the individual infant's need of vitamin C is being fully met is by determinations of the reduced ascorbic acid content of the blood plasma. According to Ingalls,[46] reduced values of ascorbic acid in the plasma from 2 to 1 mg. per hundred cubic centimeters indicate complete saturation, from 1 to 0.7 mg. normal, from 0.7 to 0.5 mg. low normal, from 0.5 to 0.3 mg. suboptimal, from 0.3 to 0.15 mg. asymptomatic scurvy and from 0.15 mg. to 0 scurvy. On the basis of combined saturation and blood tests conducted on a group of healthy infants and children, Chu and Sung [54] have proposed a similar classification based on ascorbic acid values on the plasma as follows: deficiency stage 0.17-0.41 mg., presaturation 0.54-0.8 mg., relative saturation 0.86-1.36 mg., and postsaturation 0.85-1.25 mg. These figures are in good agreement with the standard proposed by Ingalls, based on blood values alone. It will be seen that the lowest values, 0.15 and 0.17 mg. per hundred cubic centimeters, correspond to the blood content above which, according to Göthlin, the test for capillary fragility is inapplicable. Between this "physiological minimum" and the theoretic optimal is a fairly wide range with a still indefinite end point. In Ingall's opinion "it would seem logical to suppose that saturation is the truly optimal state of ascorbic acid nutrition for the infant since in the event of an increased demand or decreased supply the vitamin depot is presumably larger than in partial saturation."

Acceptance of the theory of saturation as the optimal state involves the selection of maximum rather than minimum estimates of requirements of vitamin C. Ordinarily the additional needs of the breast-fed infant and the entire needs of the artificially fed can be met by daily allowances of orange juice, tomato juice or other fruit juices without resorting to ascorbic acid, which, however, is extremely useful in cases in which fruit juices are poorly tolerated. Of particular value to the physician or hospital dietitian in calculating the equivalent quantities of various fruit juices in terms of the day's requirement of vitamin C is the recent report of the Council on Foods [55] on the ascorbic acid content of all brands of commercially canned fruit juices accepted by the Council prior to Sept. 1, 1937. With fresh orange juice of an average ascorbic acid content of about 0.5 mg. per cubic centimenter, a 50 mg. requirement would be met by 100 cc. of the juice. In comparison with this, equivalent volumes for the various canned juices studied were: canned orange juice 110 cc., lemon juice 100 cc., grapefruit juice 125 cc., tomato juice 250 cc. and pineapple juice 350 cc. These figures are only approximate and represent a rather wide range in individual values. Furthermore, the discovery of marked differences in the ascorbic acid content of varieties and strains of tomatoes [56] may be expected to raise the average of the vitamin C content of tomato juice through the selection for the commercial manufacture of tomato juice from strains of the highest vitamin C content. At present, however, it is safe practice to use the foregoing equivalents in making a substitution for orange juice in infant feeding. In large clinics with suitable laboratory facilities an even better practice, already adopted in some hospitals, is to determine the vitamin C content of each new supply of canned fruit juices for the necessary calculations in dietary prescriptions. A study of the relative costs of various fruit juices, canned and fresh, has been reported by Hawley.[57]

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[54] Chu, F. T., and Sung, C.: The Vitamin C Content of the Blood Plasma in Normal and Sick Children, Chinese M. J. **52:** 791 (Dec.) 1937.

Children.—There have been very few reports of quantitative studies on the vitamin C requirements of healthy children. This is not surprising considering the difficulty in carrying out urinary excretion studies and the possible dangers involved in subjecting children to the depletion in reserves involved in any of the test dose methods for determining requirements.

Among the subjects studied by Widenbauer,[29] using an elaborate procedure, was a healthy boy in his third year. In four out of seven tests his adjusted vitamin C consumption amounted to 22 mg., and in the other three to 21 mg. daily. These values are of special interest in terms of relative requirements, for by exactly the same methods the requirements of a grown man were 28 mg. in five tests, and 26 and 27 mg. in one test each. The very slight difference between the young child and the adult indicates that the vitamin C requirements throughout life cannot be calculated in terms of body weight alone, as was at one time suggested.[18]

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[55] 55. The Vitamin C Content of Commercially Canned Tomato Juice and Other Fruit Juices as Determined by Chemical Titration, Council on Foods, J. A. M. A. **110:** 650 (Feb. 26) 1938.

[56] 56. Maclinn, W. A.; Fellers, C. R., and Buck, R. E.: Tomato Variety and Strain Differences in Ascorbic Acid (Vitamin C) Content, Proc. Am. Soc. Hort. Sc. **34:** 543 (April) 1937. Tripp, Francis; Satterfield, G. H., and Holmes, A. D.: Varietal Differences in the Vitamin C (Ascorbic Acid) Content of Tomatoes, J. Home Econ. **29:** 258 (April) 1937.

[57] 57. Hawley, Estelle E.: The Vitamin C Content of Fruit Juices, J. Am. Dietet. A. **13:** 261 (Sept.) 1937.

In view of the customary use of cod liver oil or other vitamin A preparations in conjunction with orange juice in infant and child feeding, further observations on the same child are of interest. Immediately following the test period in which the consumption value of 21 mg. was established, he was given 2 tablespoonfuls of cod liver oil daily for two weeks, at the end of which time the values remained unchanged. Six months later, after a value of 22 mg. had been established, 5 cc. of the vitamin A concentrate "Vogan" was given daily for a week, after which a new test gave a value of only 9 mg. daily. These observations, which were substantiated on other subjects, suggest that vitamin A preparations at least do not increase the requirement of vitamin C and in particularly concentrated form may exert an indirect sparing action.

Three boys of preschool age (39, 57 and 59 months of age at the beginning of the experiment) served as subjects in what is perhaps the most detailed investigation of the vitamin C metabolism of young children thus far published in this country.[58] During twelve successive five day periods these children, who were kept under constant observation, were given weighed diets differing from period to period only in increasing and known amounts of vitamin C. With increasing intakes per kilogram of body weight the ascorbic acid retentions (intake minus output) were greater up to a certain point, beyond which there was no further increase with larger doses. The peaks in retention were reached at intakes of 7.5, 6.6 and 6.4 mg. per kilogram of body weight for the three subjects in the order of increasing weight. Retention values in two periods on these intakes were practically identical, 4.2, 3.8 and 3.6 and 4.1, 3.7 and 3.6 mg. per kilogram of body weight respectively. The somewhat higher intake and retention of the youngest child and the lower and almost identical values for the two older subjects are of interest in view of the difference of eighteen months between the ages of the youngest and next older, and only two months between this and the oldest child. If saturation is the criterion of optimal vitamin C nutrition, the intakes required for maximum retention might be considered as representing the vitamin C requirement of these children. Expressed as total daily intakes at the peak of retention, the average values in round numbers were 117, 121 and 120 mg. respectively. At this point the urinary excretion of vitamin C by two of the subjects exceeded and of the other very nearly equaled 50 per cent of the intake, another criterion of saturation.

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[58] Everson, Gladys J., and Daniels, Amy L.: Vitamin C Studies with Children of Preschool Age, J. Nutrition **12**: 15 (July 10) 1936.

There is a wide range between the Widenbauer figures of 20 and 21 mg. of ascorbic acid as the daily requirement of a child in his third year and the maximum retention or saturation values of from 117 to 123 mg. for children from 3 to 5 years of age, as calculated from these data of Everson and Daniels. Although the higher values seem unpractical if considered in terms of orange juice alone, 2 cc. of which is usually required to furnish 1 mg. of ascorbic acid, a considerable proportion of the total intake of ascorbic acid in these experiments was furnished by other foods—as would be the case in ordinary, well planned diets for growing children. In the particular diets on which maximum retentions were secured on the two extremes of intake, 116.7 and 122.8 mg. respectively, orange juice furnished 96 and 90 mg. of the total, requiring about 6½ and 6 ounces (195 and 180 cc.) respectively. While this is a considerably larger volume than the customary allowance for children, it is evidently not beyond the capacity of the preschool child.

Other studies dealing with the vitamin C metabolism of children [59] represent, for the most part, hospitalized patients and are of doubtful value in an estimation of the requirements of normal healthy children although of interest in showing the increased use or wastage of vitamin C in certain diseases.

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[59] Harris, Abbasy and Yudkin.[19] Sendroy, J., Jr., and Schultz, M. P.: Studies of Ascorbic Acid and Rheumatic Fever: I. Quantitative Index of Ascorbic Acid Utilization in Human Beings and Its Application to the Study of Rheumatic Fever, J. Clin. Investigation **15**: 369 (July) 1936. Schultz, M. P.: Studies of Ascorbic Acid and Rheumatic Fever: II. Test of Prophylactic and Therapeutic Action of Ascorbic Acid, ibid. **15**: 385 (July) 1936. Abbasy, M. A.; Hill, N. G., and Harris, L. J.: Vitamin C and Juvenile Rheumatism, with Some Observations on the Vitamin-C Reserves in Surgical Tuberculosis, Lancet **2**: 1413 (Dec. 12) 1936. Abbasy, M. A.; Harris, L. J., and Gray Hill, N.: Excretion of Vitamin C in Osteomyelitis, ibid. **2**: 177 (July 24) 1937. Keith, J. D., and Hickmans, Evelyn M.: Vitamin C Excretion in Children with Particular Reference to Rheumatic Fever, Arch. Dis. Child. **13**: 125 (June) 1938.

The studies of Abt, Farmer and Epstein [33a] and of Ingalls [33f] have shown the constancy of the ascorbic acid content of blood plasma of normal subjects at different ages and the validity of applying to children the standards previously noted. Attention has already been called to the shortened procedure recommended by Harris and Abbasy [26] for routine surveys of school children. In attempting to set up a "normal minimal" standard of urinary output of ascorbic acid more adapted to children than the 13 mg. standard of Harris, Bumbalo [60] found that the excretion was directly dependent on the intake and showed no constancy from subject to subject. Among sixteen healthy children from 5 to 13 years of age, the twenty-four hour ascorbic acid excretion ranged from 11 mg. for a boy aged 13 years whose daily diet included a glass of orange juice (about 60 cc.) to 71 mg. for a girl aged 5 years consuming four or five large oranges daily. The average daily excretion for the group was approximately 28 mg. as compared with about 8 mg. for the same number of tuberculous children in the same age range. Baumann and Rappolt [61] consider that a child is in a state of vitamin C saturation if more than 80 per cent of a test dose of 100 mg. of acorbic acid administered orally is excreted within twenty-four hours; if the excretion is between 80 and 40 per cent, a slight deficiency, and under 40 per cent a marked deficiency is indicated.

Accumulated observations by these methods and further studies of the concentration of ascorbic acid in the blood of children on diets of known ascorbic acid content should afford a better basis for estimating the vitamin C requirements of children. Meantime the chief and perhaps the only valid arguments against great liberality in vitamin C allowances for young children are the relatively high cost of orange juice and equivalent quantities of tomato juice and other fruit juices, and the possible, although exceptional, danger of acquiring a sensitivity to these juices through unusually high intakes. For older children with a greatly increased variety of foods furnishing appreciable quantities of vitamin C it is possible to provide a liberal amount of the vitamin even in low cost diets. As a final word in favor of liberal allowances, a statement by Chapin [62] at a round table discussion at the 1937 meeting of the American Academy of Pediatrics may be quoted: "Apparently the minimum amount [of vitamin C] needed to prevent scurvy is only a fraction of the desired amount for optimal health. So far as is known, it has never been possible to produce toxic symptoms by giving large doses of vitamin C."

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[60] Bumbalo, T. S.: Urinary Output of Vitamin C of Normal and of Sick Children, Am. J. Dis. Child. **55:** 1212 (June) 1938.

[61] Baumann, T., and Rappolt, L.: Untersuchungen zum C-Vitaminstoffwechsel: 3. Ueber den C-Vitaminstoffwechsel bei Kindern, Ztschr. Vitaminforsch. **6:** 24 (Jan.) 1937.

Adults.—Göthlin's earliest investigation of the physiologically indispensable requirements of vitamin C, as determined by tests for capillary fragility [2] has been repeated by Göthlin and his associates [31] with the use of ascorbic acid in place of orange juice and a mixed diet estimated to furnish only 2 mg. of ascorbic acid per person daily in place of the liquid diet of the earlier study. The subjects were again physically healthy schizophrenic adults, two males and two females, having normal gastric secretion of hydrochloric acid, blood sedimentation rate and capillary resistance. The experiment required from six to eight months for completion. At the point of final restoration to normal of the lowered capillary resistance the intakes of ascorbic acid, corrected by the addition of 2 mg. for the ascorbic acid content of the diet amounted to 0.39, 0.43, 0.44 and 0.48 mg. per kilogram of body weight for the four subjects respectively, representing a range of from 23.4 to 28.8 mg. for the total daily requirement of an adult weighing 60 Kg. These values are in surprisingly close agreement with the earlier estimates of from 21 to 30 mg. as calculated from the orange juice required in Göthlin's first experiments and of from 19 to 27 mg. as estimated from guinea pig requirements for the same degree of protection. Commenting on these values as related to dietary recommendations, the authors state:

> It will be realized that the daily supply should be more generous than the figures indicate. The supply should no more be limited to what has proved to be the indispensable minimum

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[62] Chapin, H. D.: Vitamins C and D (in Round Table Discussion on Vitamins), J. Pediat. **12**: 529 (April) 1938.

> than the supply of protein in the dietary for man should be limited to the minimum intake sufficient to maintain nitrogen balance.

However, no estimate is given for the lower limit of optimal daily intake on the ground that any investigation of this question

> should take into consideration the circumstance that the supply of vitamin C which has been usual for generations is, owing to differences in obtainable food material and consequently in diet, entirely different for the people who live in different latitudes, a circumstance which may also possibly have affected their vitamin requirements.

It is significant that by an entirely different method, but one which may also be interpreted as yielding minimum rather than optimal values, Widenbauer[29] obtained values of about 28 mg. as his own daily requirement over a total period of 210 days and that of a nonpregnant woman. As no account was taken of the vitamin C content of the diet, which was low but not absolutely free from ascorbic acid, the actual consumption of vitamin C was undoubtedly somewhat higher than the figures given.

In experiments conducted by van Eekelen[30] on himself following the method previously outlined, which may be expected to give higher values than either the Göthlin or the Widenbauer method, the requirement or "daily use" of ascorbic acid amounted to 34 mg. after a depletion period of ninety-four days and to 63 mg. after a period of only twenty-seven days. In van Eekelen's opinion, the daily requirement of ascorbic acid is adapted to the existing storage of the vitamin. If the diet is customarily low in vitamin C or if, as in the case of some of his experiments, there has been a prolonged depletion period, the requirement or "daily use" of the vitamin is lower than if the diet has been richer in the vitamin or there has been only a short depletion period. The selection of the upper value (63 mg.) as his daily requirement was presumably based on a consideration of his customary dietary habits. The same idea of decreased requirements with prolonged subsistence on diets low in vitamin C has been brought out by Wachholder,[63] who also considers it unwise to recommend the lowest values that may be obtained because they provide no margin of safety for increased use of the vitamin in infections and for the possible increased requirements of muscular work either in manual labor or in exhaustive sports.

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[63] Wachholder, K.: Ueber die Vitamin C-Bilanz des Menschen: IV. Einschränkung des Bedarfes bei mangelhafter Zufuhr und deren ernährungsphysiologische Bedeutung, Klin. Wchnschr. **17**: 5 (Jan. 1) 1938.

With due recognition of the influence of the past dietary history on vitamin C requirements, a factor also recognized by Göthlin as already noted, the van Eekelen method still seems lacking in precision. Göthlin [31] has criticized the indefiniteness in the end point of the test as follows:

> Without an objective reason for deciding the duration of the period of abstinence, it appears that every preconceived view as to the requirements of ascorbic acid in man could find confirmation by means of an investigation in accordance with van Eekelen's principle provided that the length of the period of abstinence is adapted to suit the preconceived view.

In two other studies by essentially the same method [64] requirement values were obtained for four adult subjects equivalent to 0.70, 0.78, 0.83 and 0.83 mg. respectively per kilogram of body weight. For the adult weighing 60 Kg. the range in total daily requirement is from 42 to 50 mg., or nearly twice as much as the indispensable minimum requirement, whether determined by the Göthlin test for capillary fragility or by the saturation method of Widenbauer.

The saturation method employed by Hauck has not yet been tested on a sufficient number of subjects to warrant definite conclusions, but preliminary results are of considerable interest. For the six subjects (college graduate students and faculty) on whom at least three separate preliminary saturation tests had been made at the time of writing, the average responses in urinary excretion of ascorbic acid to a 400 mg. test dose administered after the subjects had received 200 mg. of ascorbic acid daily for six days in addition to the vitamin C furnished by a diet not absolutely free from vitamin C ranged from 229 to 274 mg. with two of the subjects giving no individual response above 249 mg.

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[64] van Wersch, H. J.: Determinations of the Daily Requirements for Ascorbic Acid of Man, Acta brev. neerland. **6**: 86 (July 4) 1936. Heinemann, M.: On Human Requirements for Vitamin C Under Different Conditions, ibid. **6**: 144 (Oct. 15) 1936. Heinemann, M.: I. On the Relation Between Diet and Urinary Output of Thiosulfate (and Ascorbic Acid): II. Human Requirements for Vitamin C, Biochem. J. **30**: 2299 (Dec.) 1936.

and two none lower than 240 mg. These values afford another illustration of differences in the saturation or retention capacities of different individuals. In four subjects for whom fairly clearcut results were obtained in the final adjustment in dosage of ascorbic acid to the smallest amount producing excretion values comparable to the individual standards established in the preliminary tests, the estimated requirements were equivalent to 1.18, 1.24, 1.33 and 1.62 mg. per kilogram of body weight or from a little over 70 to nearly 100 mg. as the daily requirement for maintaining complete saturation in an adult weighing 60 Kg.

Thus for adults as well as for infants and children the range of estimated requirements from the physiologically indispensable minimum to complete saturation is extremely wide—from 25 or 30 mg. to 100 mg. daily or from about 0.4 to 1.6 mg. per kilogram of body weight—a fourfold increase from the absolute minimum to what might be called luxus consumption. Whatever may be the uncertainty about the upper limits of requirement, there seems no doubt that the 30 mg., as stated by the Technical Commission on Nutrition of the Health Organization of the League of Nations[44] "to be a curative dose for adults suffering from scurvy," should be considered only as the minimum for protection against scurvy and not as the optimum for satisfactory nutrition. For this even the frequently recommended allowance of 50 mg. should probably be considered as the barely adequate rather than the optimal requirement, with considerably larger quantities desirable when economically possible. There is some indication that a particularly generous margin of safety should be the rule in allowances of vitamin C for old people.[65] As will be noted in the following section, pregnant and lactating women should have at least twice as much vitamin C as would meet the ordinary requirements of nonpregnant women.

A report from the Massachusetts and Rhode Island state colleges[66] covers urinary excretion studies on more than 200 freshmen and forty junior women students. The Massachusetts subjects included two groups of about seventy students each tested at the beginning and near the close of their first year at college and the Rhode Island subjects about sixty freshmen and forty juniors tested the same year. The range in the "resting level" of urinary ascorbic acid was very wide in each group but strikingly similar for the three groups of freshmen students as reflected in the graphic array of individual figures and also in values of 15 mg. (Rhode Island), 20 mg. (Massachusetts, 1936) and 22 mg. (Massachusetts, 1937) representing the average daily excretion of ascorbic acid of the subjects in each group. Corresponding averages for the Rhode Island juniors and practically the same groups of Massachusetts students tested in the spring of their freshman year were 28, 33 and 27 mg. respectively. These higher averages as well as decidedly higher values for some of the individual students suggest aroused interest in the selection of foods of higher intake of vitamin C as the result of individual or group instruction.

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[65] Harris, Abbasy and Yudkin.[19] Gander and Niederberger.[27]

[66] Mitchell, Helen S.; Merriam, Oreana A., and Batchelder, Esther L.: The Vitamin C Status of College Women as Determined by Urinary Excretion, J. Home Econ., to be published.

In the first period in the aforementioned study about 70 per cent of the Massachusetts and 85 per cent of the Rhode Island students had excretion values of 20 mg. or less, while in the second period this figure dropped to 46 and 47 per cent for the Massachusetts subjects and 50 per cent for the Rhode Island juniors. Unpublished data from comparable studies with even greater uniformity in technic on groups of students at the state colleges of Montana, Oregon, Washington and Utah [67] show a similar wide range in individual values. Rough calculations indicate that excretion values of 20 mg. or less were the rule in about 33 per cent of the Montana, 35 per cent of the Oregon, 30 per cent of the Utah and 32 per cent of the Washington group. If there is any significance in the statement of the Harris group [19] that an excretion of 20 mg. indicates a moderately low intake of vitamin C, it would seem that about one third of the young women college students in the Northwest and about three fourths of similar students in the Northeast with no particular dietary instruction were on a none too liberal vitamin C intake.

In these cooperative studies smaller numbers of subjects are being given saturation tests, and an effort is also being made to correlate the estimated vitamin C content of the individual diets with the excretion values, but informaton is not yet available on these points.

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[67] Richardson, Jessie E.; Fincke, Margaret L.; Todhunter, E. Neige, and Brown, Almeda P.: Personal communications to the author.

Pregnant and Lactating Women.—All evidence points to a greatly increased need of vitamin C in pregnancy, which continues through the lactation period. In the investigation of Widenbauer,[29] to which frequent reference has been made because of the comparative value of his data, a nonpregnant woman with a minimum requirement or "daily use" of 28 mg. was found to need 71 mg. in the third and 67 mg. in the eighth month of pregnancy. The slight drop in the later period may not be significant, but the two values in comparison with the nonpregnant value indicate a more than doubled requirement during pregnancy. Following the same method with a group of healthy women in the eighth and ninth months of pregnancy, Gaehtgens [68] obtained the rather wide range in values of from 33 to 64 mg. and recommended not less than 100 mg. daily as a safe requirement for pregnancy. As his values were obtained by a method which gives only about 28 mg. as the ordinary adult requirement, the additional allowance for pregnancy is very generous. In Gaehtgens' opinion, the increased vitamin C requirement during pregnancy is due not to increased metabolism but to the fetal and placental requirements for storage. Evidence of increased requirements during pregnancy has also been shown by Neuweiler [69] in urinary excretion tests following the intravenous injection of 200 mg. of ascorbic acid. More than half of a group of apparently healthy pregnant women were found by Gaehtgens and Werner [70] to have a significant saturation deficit as indicated by the increased length of time required for saturation according to the Jezler and Kapp criteria.[21]

It is obvious that the nursing mother needs enough vitamin C to meet her own requirements and to maintain a satisfactory concentration of the vitamin in her milk. The various studies on the requirements of vita-

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68. Gaehtgens, G.: Der Tagesverbrauch an Vitamin C in der Schwangerschaft, Arch. f. Gynäk. **164:** 571 (Aug. 26) 1937.

69. Neuweiler, W.: Ueber den Bedarf an Vitamin C während Gravidität und Lactation, Klin. Wchnschr. **14:** 1793 (Dec. 14) 1935.

70. (*a*) Gaehtgens, G., and Werner, E.: Das Vitamin C-Defizit in der Gravidität, Arch. f. Gynak. **163:** 475 (March 5) 1937; (*b*) Zur Frage des Vitamin C-Defizits in der Gravidität und während der Lactation, Klin. Wchnschr. **16:** 843 (June 12) 1937.

min C during lactation [71] have shown that under favorable conditions breast milk can be enriched to values considerably above the average but that there is an upper limit which cannot be exceeded. As Baumann [72] has expressed it, there is no lower threshold for the secretion of ascorbic acid from the blood into the milk and the process may go on until the maternal organism is greatly depleted, but there is an upper limit of concentration at about 8 mg. per hundred cubic centimeters of milk. Conversely, there is no upper limit of excretion of ascorbic acid in the urine but a lower one of about 0.1 to 0.14 mg. per hundred cubic centimeters of blood. Baumann and Rappolt [50] have calculated that for a milk production of 800 cc. a day with an ascorbic acid content of at least 5 mg. per hundred cubic centimeters at least 50 mg. of ascorbic acid should be added to the daily requirement of the nonpregnant woman.

A distinction has been pointed out by Gaehtgens and Werner [73] between the effects of a deficiency of vitamin C in pregnancy and in lactation. When the requirements are not met during gestation, the maternal organism becomes depleted at the expense of the fetus; whereas in lactation the maternal organism is protected and the infant suffers through decreased content of ascorbic acid in the milk. If this theory is sound, it would seem advisable to be more insistent on recommending a greatly increased intake of vitamin C during pregnancy than in lactation and rely more in the latter period on supplementing the probable deficiencies in the breast milk by giving orange juice or other easily taken and readily assimilable sources of vitamin C directly to the infant at a very early age. However, no harm and undoubtedly much benefit will accrue to the nursing mother if her vitamin allowance is doubled during the period of considerable drain on her reserves.

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[71] Neuweiler.[69] Chu, F. T., and Sung, C.: Effect of Vitamin C Administration on Vitamin C of Milk and Urine of Lactating Mothers, Proc. Soc. Exper. Biol. & Med. **35:** 171 (Oct.) 1936. Widenbauer and Kühner.[49e] Baumann and Rappolt.[50] Gaehtgens and Werner.[70b]

[72] Baumann, T.: Untersuchungen über den C-Vitaminstoffwechsel bei laktierenden Frauen und über den Grad der physiologischen und pathologischen C-Vitaminsättigung des menschlichen Organismus, Jahrb. f. Kinderh. **150:** 193, 1937; Nutrition Abstr. & Rev. **7:** 1083, 1937.

[73] Gaehtgens, G., and Werner, E.: Vitamin C-Belastungen bei Stillenden Wöchnerinnen, Arch. f. Gynäk. **165:** 63, 1937.

FACTORS AFFECTING REQUIREMENTS OF VITAMIN C

While it is beyond the scope of this paper to discuss the requirements of vitamin C in illness, and the studies that have been reviewed in an attempt to arrive at some decision concerning requirements have been confined to those dealing with presumably physiologically healthy subjects, no greater argument can be given in support of liberality in allowances than to call attention to the increased use or disappearance of ascorbic acid which has been observed under various conditions of heightened metabolic activity such as heavy manual labor or violent exercise [25] and in certain febrile conditions, notably tuberculosis.[74] In fact it has even been suggested that ascorbic acid metabolism may be an index of total metabolism and thus serve as a general diagnostic guide.

VITAMIN C IN HUMAN DIETS

With the ascorbic acid content of any individual food in its natural state varying as widely as has been shown in two recent critical compilations of the literature,[75] and with the uncertain losses that may occur in such foods before their final consumption, it has seemed useless to attempt to estimate requirements from records of food consumed by groups of presumably healthy persons of different ages, nor can such records be expected to give accurate data as to the actual consumption of ascorbic acid in comparison with estimated requirements. It is, nevertheless, of some interest, as indicating trends in consumption, to study the accompanying table, a companion to the one presented for vitamin A in a previous paper of this series.[76]. With all due allowance for the sources of error in such calculations, it is of significance that as the weekly expenditure for food increased the estimated ascorbic acid content of the diet increased almost without exception. When it is considered that in the low cost diets the proportion of ascorbic acid furnished by orange juice was probably much less than in diets of higher cost and that consequently a greater deduction should be made for losses of the vitamin before consumption, it will be seen that certainly the lowest cost diet, and probably the next higher (except for the farm group) furnished less than a safe allowance of vitamin C and that only in the two highest cost

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[74] Martin, G. J., and Heise, F. H.: Vitamin C Nutrition in Pulmonary Tuberculosis, Am. J. Digest. Dis. & Nutrition **4:** 368 (Aug.) 1937.

[75] Daniel and Munsell.[52] Boas Fixsen, Margaret A., and Roscoe, Margaret H.: Tables of the Vitamin Content of Human and Animal Foods. Nutrition Abstr. & Rev. **7:** 823 (April) 1938.

[76] Booher, Lela E.: Vitamin A Requirements and Practical Recommendations for Vitamin A Intake, J. A. M. A. **110:** 1920 (June 4) 1938.

Estimated Ascorbic Acid Content of Diets of Families Not on Relief, 1935-1936 (in Milligrams per Requirement Unit) [*]

Region and Degree of Urbanization	Average[†] in Milligrams per Adult per Day[‡] for Families Spending[§] Weekly per Head for Food				
	$1.25-$1.87	$1.88-$2.49	$2.50-$3.12	$3.13-$3.74	$3.75-$4.37
City[‖]					
North Atlantic.....	40.0	57.5	70.0	87.5	97.5
East North Central	47.5	57.5	75.0	77.5	107.5
East South Central	50.0	67.5	85.0	....	....
Pacific..............	70.0	77.5	95.0	107.5	115.0
Village[#]					
North Central......	40.0	57.5	77.5	77.5	....
Southeast..........	40.0	52.5	72.5	95.0	92.5
Pacific..............	50.0	77.5	87.5	105.0	100.0
Farm[¶]					
North Central......	66.5	70.0	90.0	100.0	....
Southeast..........	55.0	70.0	80.0	....	....
Pacific..............	....	83.5	106.0	....	....

[*] Data furnished by Dr. Hazel K. Stiebeling, Senior Food Economist, Bureau of Home Economics, U. S. Department of Agriculture.

[†] These averages represent the value of the uncooked food. They are higher, therefore, than the value of food actually eaten.

[‡] The following scale of family coefficients was employed in evaluating the ascorbic acid intake for families of different types in terms of an adult:

Adults, 20 years and over..................................	1.0
Boys 16-19 years..	1.2
Boys 13-15 years..	1.0
Boys 11-12 years, girls 14-19 years........................	0.9
Boys 9-10 years, girls 11-13 years..........................	0.8
Boys 4-8 years, girls 4-10 years.............................	0.7
Children under 4 years.......................................	0.7

[§] Adjusted to 1935 retail price levels.

[‖] Families of employed workers. Field work conducted by the U. S. Bureau of Labor Statistics.

[#] All occupational groups.

[¶] Farm operators; farm-furnished food valued at farm prices.

groups could the intake be considered to represent a very generous allowance.

A point of interest is the consistently higher ascorbic acid content of the diets at all cost levels in the Pacific than in the North Atlantic region for the city families, the only group for which data are reported for both of these regions. These differences are brought out even more strikingly in the detailed report on the diets of families of employed wage earners and clerical workers in cities.[77] As many as 45 per cent of these families in the North Atlantic region bought foods supplying less than 37.5 mg. of ascorbic acid per requirement unit a day, whereas none of the Pacific city families had diets so low in vitamin C. With expenditures for food ranging from $2.50 to $3.12 per person weekly, considered representative of the moderately well-to-do, about 37 per cent of the families in the North Atlantic group and 70 per cent in the Pacific group bought food furnishing more than 75 mg. of ascorbic acid (uncorrected) a day. These figures are entirely consistent with the urinary excretion data for groups of college women in these areas for, as noted earlier, low excretion values for ascorbic acid (presumably indicative of low intake) were the case in from 75 to 80 per cent of the North Atlantic group and from 30 to 35 per cent in the Pacific group.

SUMMARY AND CONCLUSIONS

A positive statement of human requirements is difficult to make because of uncertain or unsatisfactory methods for its determination in the living organism, lack of agreement as to the most satisfactory state of nutrition with respect to this vitamin and individual differences in requirements.

Based on the methods that have been followed, three levels of requirement may be considered in terms of the minimum quantities of ascorbic acid required: (1) to prevent the slightest decrease in capillary resistance or to maintain uniform excretion of the vitamin barely above that on a vitamin C-free diet; (2) to maintain uniform excretion after a depletion period following a preliminary saturation period, and (3) to maintain the tissues in a state of saturation as determined by various modifications of the test dose method. These levels of requirement may conveniently be termed the physiologically indispensable, adequate, and saturation or luxus consumption values respectively.

The estimated requirements [78] for various age groups as reviewed lie within the following limits of absolute values: infants, from about 8 mg. (newly born) to 50 mg. daily; children, from 22 to 100 mg. or more; adults, from 28 to 100 mg. or more. With due allowance for unlike methods followed in their determination, these values may be considered to represent roughly the range from the physiologically indispensable minimum to saturation or luxus consumption. Values of about 20 mg. daily for infants, 40 mg. for children and from 50 to 60 mg. for adults may be considered as tentative estimates for the middle or barely adequate consumption level, with but little margin of safety or allowance for individual variations in requirement.

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[77] Stiebeling, Hazel K., and Phipard, Esther F.: Diets of Families of Employed Wage Earners and Clerical Workers in Cities, U. S. Dept. Agr. Circ. 507, 1938.

[78] Perhaps it should be emphasized again that these estimates are based largely on indirect evidence and greatly exceed estimates based on evidence of harmful effects of deficiency in the human organism.—Ed.

Requirements have also been estimated in terms of body weight: infants from 3 to 8 mg., children from 6.4 to 7.5 mg. and adults from 0.7 to 1.6 mg. per kilogram of body weight. While the data are far too incomplete to warrant separation of these values into the three levels or to permit of definite conclusions concerning the rate of decrease in requirement per unit weight with increasing age, a comparison of the absolute and relative values for different ages suggests that vitamin C requirements are related both to body weight and to metabolic rate, the effect of the latter being the more pronounced.

It has been stated that "saturation is the truly optimal state of ascorbic acid nutrition for the infant since in the event of an increased demand or decreased supply, the vitamin depot is presumably larger than in partial saturation." [33f] The same argument would seem to be valid for the growing child. Since vitamin C is intimately concerned with cellular metabolism, liberality in allowance throughout the entire period of growth seems logical. There is still considerable difference of opinion concerning the satisfactory allowance for the adult. While it may be safe to accept the middle level of requirement, the upper level of luxus consumption is more in keeping with the policy of providing a generous margin of safety in most dietary allowances. In pregnancy and lactation with their increased demands, the margin of safety afforded by the upper level of requirement is most essential, and this may also hold for elderly persons.

CHAPTER XXII

VITAMIN C

PHARMACOLOGY AND THERAPEUTICS

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Vitamin C is specifically involved in the prevention or treatment of scurvy. Since this factor was isolated, its structure determined and the pure substance synthesized, considerable advances in our knowledge of the physiology of vitamin C have been made and some evidence of the value of this substance in conditions other than scurvy has been developed. Exact knowledge of the pharmacologic action of vitamin C is still rather limited, but it is growing rapidly because of the development of chemical methods for estimating vitamin C in foods and body tissues and fluids. The present review is an attempt to summarize this knowledge as well as the present status of our knowledge of the therapeutic usefulness of vitamin C.

AVAILABLE PREPARATIONS OF VITAMIN C

It must not be forgotten that vitamin C is an accessory food substance and that ordinarily foods are relied on for a supply of this essential factor. As described in the paper on sources of vitamin C,[1] there are some foods which are especially potent. Chief among these are the citrus fruit juices, which long have been used in the prevention and treatment of scurvy. Fresh orange juice contains on the average from about 40 to 60 mg. of vitamin C per hundred cubic centimeters. The daily requirements of an adult are considered to be about 25 mg. for the prevention of scurvy and more than that for normal requirements. The exact requirements for normal health are not known but nutritionists have estimated that a standard allowance for an adult would

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From the Northwestern University Medical School.

[1] Bessey, Otto A.: Vitamin C: Methods of Assay and Dietary Sources, J. A. M. A. **111:** 1290 (Oct. 1) 1938.

be from 600 to 1,200 international units, equivalent to from 30 to 60 mg. of vitamin C. Canned tomato juice is also a rich source of vitamin C, as are the canned citrus fruit juices.[2]

It is customary to give vitamin C to all babies early in life. This is usually administered in the form of fresh orange juice, beginning even as early as the third week of life with a teaspoonful and, if well tolerated, increasing gradually until the baby is taking about 2 ounces daily by the third month. On this basis the infant will be receiving up to about 25 mg. or more of vitamin C a day. Other sources of vitamin C may be substituted for the fresh orange juice and this substitution should be on the basis of the vitamin C content.

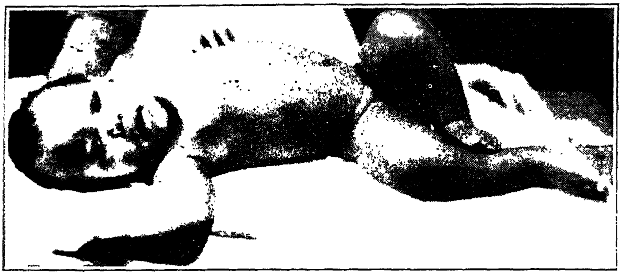

Fig. 1.—Infant with scurvy. Note particularly edema of the legs and expression of face.

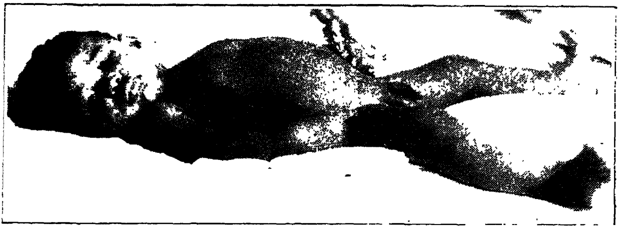

Fig. 2.—Infant after treatment with ascorbic acid, showing recovery.

Synthetic preparations of crystalline vitamin C are now available. Ascorbic acid is freely soluble in water and may be administered orally in tablet form or dissolved in water. For parenteral use, either intramuscularly or intravenously, Fisher and Leake[3] have recommended that ascorbic acid be dissolved in sterile water and neutralized with one-half its weight of sodium bicarbonate immediately before injection. If this procedure is followed, the vitamin will not lose in potency and local reaction will be avoided.

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[2] The Vitamin C Content of Commercially Canned Tomato Juice and Other Fruit Juices as Determined by Chemical Titration: A Report of the Council on Foods, J. A. M. A. **110:** 650 (Feb. 26) 1938.

Before the isolation of the crystalline vitamin, doses larger than 500 mg. daily (approximate content of 1 quart of orange juice) were impracticable. We have noted in some of our experimental work that certain persons will not tolerate even 1 pint of orange juice daily. Since the crystalline vitamin has become available, it may be used to supplement the dietary intake or to be given in large doses.

The crystalline vitamin has an indicated therapeutic value in infant feeding under certain conditions. There are some infants who are allergic to or intolerant to fruit juices and other foods rich in vitamin C. This intolerance or sensitivity is presumably due to the proteins of the juices or foods containing the natural vitamin, and in such cases the crystalline vitamin may be advantageously substituted. The substitution of the crystalline vitamin for the natural food sources may also have a therapeutic indication in certain acute and chronic gastrointestinal diseases in which adequate absorption of vitamin C from natural food sources is altered or interfered with.

In certain of the conditions to be discussed, relatively large doses of crystalline vitamin C are required to obtain therapeutic effects. Although the normal daily human requirements have been estimated to be from 15 to 40 mg. or even up to 60 mg.,[4] it is difficult to explain why certain therapeutic effects can be obtained only by administering doses of from ten to twenty-five times this amount. Our present state of knowledge is so limited that a precise statement of what constitutes proper dosage is not practicable. In New and Nonofficial Remedies it has been suggested that crystalline vitamin C be administered in quantities of 10 mg. for the prevention of infantile scurvy. "The therapeutic dose is about 30 mg. daily, although many physicians prefer to administer larger amounts to adults." [5]

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[3] Fisher, B. H., and Leake, C. D.: The Parenteral Administration of Cevitamic Acid (Ascorbic Acid) Solution, J. A. M. A. **103**: 1556 (Nov. 17) 1934.

[4] Smith, Sybil L.: Vitamin C Nutrition and Requirements, J. A. M. A., to be published.

One question raised with regard to the higher dosages that are possible with crystalline vitamin C is the possibility of toxicity. It is rather generally accepted that vitamin C is nontoxic when administered in moderate doses to the human being and excess amounts are excreted. Repeated doses of as much as from 1 to 6 Gm. have been administered orally and intravenously to adults, with no evidence of toxic action.[6] Friedman, McGoey and Ralli [7] in a study of vitamin C clearance have repeatedly obtained plasma concentrations ranging from 5 to 22 mg. per hundred cubic centimeters (normal values are from 0.7 to 1.5 mg.) with no evidence of toxicity. However, Schade [8] observed vagotonic symptoms in growing children at the height of the vitamin C action (fatigue, exhaustion, slow pulse, increased intestinal peristalsis). Widenbauer [9] noted vagotonic symptoms such as dermographia, hyperemia, erythema, increased peristalsis and bradycardia in infants on maximum doses of vitamin C. These symptoms were not attributed to a hypervitaminosis but to drug sensitivity or idiosyncrasy to ascorbic acid. In the guinea pig Randoin [10] has noted that, if a scorbutic animal is given relatively large quantities of vitamin C, the animal may lose weight and the symptoms of scurvy reappear.

FUNCTIONS AND PHARMACOLOGIC ACTIONS

The chief function assigned to vitamin C in the body is concerned with the formation of colloidal intercellular substances.[11] The intercellular substances which appear to be regulated by vitamin C are of mesenchymal origin

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[5] New and Nonofficial Remedies, Chicago, American Medical Association, 1938, p. 472.

[6] Personal clinical experience.

[7] Friedman, G. J.; McGoey, C., and Ralli, E. P.: Personal communication to the authors; abstract, Proc. Am. Physiol. Soc. Bull., 1938.

[8] Schade, H. A.: Beitrag zur Frage des Einflusses von Vitamin C (1-Ascorbinsäure) auf Pigmentierungsvorgange, Klin. Wchnschr. **14:** 60 (Jan. 12) 1935.

[9] Widenbauer, F.: Toxische Nebenwirkungen von Ascorbinsäure—C Hypervitaminose? Klin. Wchnschr. **15:** 1158 (Aug. 15) 1936.

[10] Randoin, G. L.: On the Necessity for a Biological Supervision of Food with Special Reference to Its Vitamin Content, Quart. Bull. Health Organ., League of Nations **5:** 493 (Sept.) 1936.

[11] Wolbach, S. B.: Pathologic Changes Resulting from Vitamin Deficiency, J. A. M. A. **108:** 7 (Jan. 2) 1937.

—the collagen of all fibrous tissue structures, all nonepithelial cement substances, including the intercellular substance of the capillary wall, dentin, cartilage, and the matrices of bone. It has also been suggested that vitamin C may function as a respiratory catalyst, aiding cellular respiration by acting as a hydrogen transport. The theory suggesting this function has received support in relation to plant and vegetable life[12] but has not been proved for animal tissue.

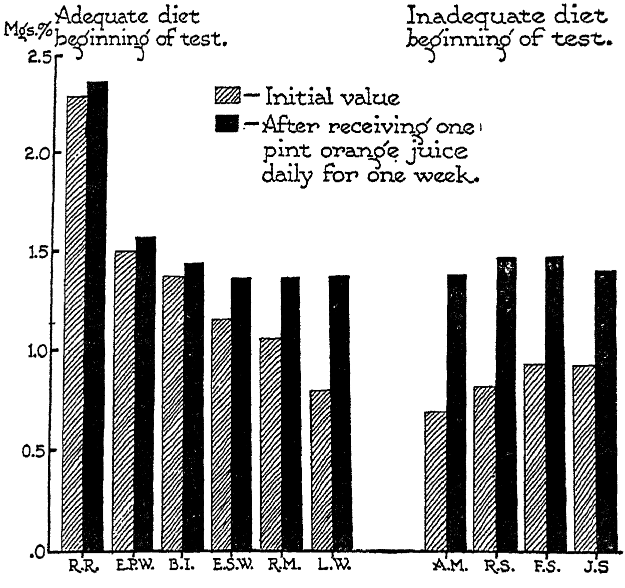

Chart 1.—Vitamin C content of the blood as influenced by diet: ascorbic acid values of young adults.

It has been shown that man, the guinea pig and the primates are incapable of synthesizing vitamin C. In tissue slice experiments, Widenbauer and Korschorreckt[13] reported an increase in vitamin C, as measured by iodine titration, when slices of intestine from the mouse or rat, animals which are capable of synthesizing vitamin C, are incubated in a sterile dextrose-salt medium. When slices of liver, spleen, stomach or brain were used, no evidence of vitamin C formation could be found. These authors concluded that animals which do not require a dietary supply of vitamin C synthesize ascorbic acid from dextrose in the intestine.

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[12] Szent-Györgyi, Albert: On the Function of Hexuronic Acid in the Respiration of the Cabbage Leaf, J. Biol. Chem. **90:** 385 (Jan.) 1931.

[13] Widenbauer, F., and Korschorreckt, K.: Ueber die Bildung von Vitamin C mit überlebenden Gewevssnitten im Reagensglas, Biochem. Ztschr. **291:** 209, 1937.

Occurrence in Body Fluids.—Vitamin C is excreted in the urine, the amounts varying with the dietary intake.[14] The level of vitamin C in the blood plasma also varies directly with the dietary intake [15] (chart 1). Different clinical methods may give different values.[15a]

Our own work leads us to believe it is important to omit the use of potassium cyanide as a stabilizer of ascorbic acid; [15b] recovery values in the titration methods,[15c] support this fact as well as the wide acceptance by many investigators of our methods without further modification.[15d] Low blood plasma values found in man have been associated with active scurvy.[16] In the scorbutic guinea pig the lowest plasma values have occurred just before death.[17] According to Borsook,[18] practically all the ascorbic acid in plasma and serum is in the reduced state.

Plaut and Bülow [19] have observed a direct relationship between the vitamin C content of the cerebrospinal fluid and the dietary intake, which corresponds to the observations on blood plasma. Stuteville [20] noted the presence of vitamin C in saliva. Abt, Farmer and Epstein[21] measured the ascorbic acid content of saliva obtained from students, before and after diets supplemented by vitamin C. No definite relationship could be established between the dietary intake and the salivary content. The salivary level was also found to be independent of the level in the blood. According to Zimmet and Dubois-Ferrière,[22] the concentration of vitamin C in the saliva of children increased progressively from a level of 0.04 mg. per hundred cubic centimeters at 4 years of age to 0.11 mg. per hundred cubic centimeters at 16 years.

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[14] Harris, L. J.; Ray, S. N., and Ward, Alfred: The Excretion of Vitamin C in Human Urine and Its Dependence on the Dietary Intake, Biochem. J. **27**: 2011 (Nov.) 1933. Harris, L. J., and Ray, S. N.: Diagnosis of Vitamin C Subnutrition by Urine Analysis, with Note on Antiscorbutic Value of Human Milk, Lancet **1**: 71 (Jan. 12) 1935. von Euler, Hans, and Klussmann, E.: Physiologische Versuch über Vitamin C (Ascorbinsäure) und Redukton, Ztschr. f. physiol. Chem. **217**: 167 (April) 1933. Johnson and Zilva.[34]

[15] Farmer, C. J., and Abt, A. F.: Ascorbic Acid Content of Blood, Proc. Soc. Exper. Biol. & Med. **32**: 1625 (June) 1935; Determination of Reduced Ascorbic Acid in Small Amounts of Blood, ibid. **34**: 146 (March) 1936. Greenberg, L. D.; Rinehart, J. F., and Phatak, N. M.: *Studies on Reduced Ascorbic Acid Content of the Blood Plasma, ibid.* **35**: 135 (Oct.) 1936.

[15a] Bessey, Otto A.: Vitamin C.: Methods of Assay and Dietary Sources. See Chapter XX.

[15b] Farmer, C. J., and Abt, A. F.: The Invalidation of Plasma Ascorbic Acid Values by the Use of Potassium Cyanide, Proc. Soc. Exper. Biol. & Med. **38**: 399 (April) 1938.

[15c] Farmer, and Abt.[15]

[15d] Present Status of Clinical Tests for Vitamin Deficiency, Am. J. Pub. Health, Supplement, **29**: 42 (Feb.) 1939. Friedman, G. J., Rubin, S. H., and Kees, W.: Effect of Addition of KCN to Whole Blood on Indophenol-Reducing Power of Plasma, Proc. Soc. Exper. Biol. & Med. **38**: 358 (April) 1938.

[16] Abt, A. F., and Epstein, I. M.: Cevitamic Acid in the Treatment of Infantile Scurvy, J. A. M. A. **104**: 634 (Feb. 23) 1935.

[17] Abt, A. F.: Cevitamic Acid of the Blood Plasma, Am. J. Dis. Child. **54**: 682 (Sept.) 1937.

[18] Borsook, Henry; Davenport, H. W.; Jeffreys, C. E. P., and Warner, R. C. P.: Oxidation of Ascorbic Acid, J. Biol. Chem. **117**: 237 (Jan.) 1937.

[19] Plaut, F., and Bülow, M.: Weitere untersuchungen über das C-Vitamin im Gehirn und im Liquor cerebrospinalis, Ztschr. f. d. ges. Neurol. u. Psychiat. **152**: 84, 1935.

[20] Stuteville, O. H.: Presence of Vitamin C in Saliva, Proc. Soc. Exper. Biol. & Med. **32**: 1454 (June) 1935.

The vitamin C content of sweat,[23] aqueous humor[24] and gastric juice[25] has been determined. A study of the occurrence of vitamin C in the feces has been made in our laboratory.[26] In the normal individual not over 6 to 10 mg. daily are excreted, even with oral feeding of large amounts. Under abnormal conditions (diarrhea, colitis and the like) the amount depends largely on the number and type of stools. Ishibashi[27] has reported studies on the vitamin C content of the feces of infants. According to him, peroral administration of the vitamin produced no fecal rise.

Absorption and Excretion.—Vitamin C appears to be selectively absorbed by the tissues of the intestinal tract,[28] principally the small intestine. Klodt[29] believes that bile may aid absorption. In achlorhydria, blood plasma levels are usually lower than normal. This may be caused by decreased absorption of active vitamin C from the intestine, which in turn is caused by a lessened acidity of the intestinal contents or by a a changed bacterial flora. In support of the latter view Kendall and Chinn[30] have shown that ascorbic acid is destroyed by certain bacteria isolated from stomach and intestinal contents of achlorhydric patients. Further evidence of the loss of vitamin C activity prior to absorption has been afforded by the observation of Wilder and Wilbur [31] that variable amounts of vitamin C are lost in the stool or destroyed in the gastrointestinal tract even under normal conditions. Hou [32] found that ascorbic acid administered by subcutaneous injection was twice as effective as that given by mouth in the protection of guinea pigs against scurvy. He has suggested as the probable explanation that part of the ascorbic acid administered orally is lost in the gastrointestinal tract. Until definite information is available to the contrary, it must be assumed that not only are there individual variations in absorption of vitamin C from the intestinal tract but also under certain conditions the absorption may be definitely abnormal.

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[21] Abt, A. F.; Farmer, C. J., and Epstein. I. M.: Normal Cevitamic (Ascorbic) Acid Determinations in Blood Plasma and Their Relationship to Capillary Resistance, J. Pediat. **8:** 1 (Jan.) 1936.

[22] Zimmet. D., and Dubois-Ferrière, H.: Les variations du pouvoir reducteur (vitamin C) de la saliva chez l'homme selon l'age, Compt. rend. Soc. de biol. **124:** 103, 1937.

[23] Cornbleet, Theodore; Klein, R. I., and Pace, E. R.: Vitamin C Content of Sweat, Arch. Dermat. & Syph. **34:** 253 (Aug.) 1936.

[24] Rosner, Lawrence and Bellows, John: Ascorbic Acid Oxidase in Determining Vitamin C in Lens and Aqueous Humor, Proc. Soc. Exper. Biol. & Med. **34:** 493 (May) 1936. Buschke, W.: Blutzirkulation im Auge und Vitamin C, Arch. f. Augenh. **109:** 691, 1936.

[25] Peters. G. A., and Martin, H. E.: Ascorbic Acid in Gastric Juice, Proc. Soc. Exper. Biol. & Med. **36:** 76 (Feb.) 1937.

[26] Farmer, C. J., and Chinn, Herman: Unpublished data.

[27] Ishibashi, T.: Vitamin C of the Urine and Feces of Newly Born Infants, Acta paediat., Japan. **43:** 187 (Feb.) 1937.

[28] Zilva, S. S.: Ascorbic Acid Content of Intestine of Guinea Pig, Biochem. J. **29:** 100 (Nov.) 1935.

[29] Klodt, W.: Ueber den Einfluss der Galle auf die Vitamin C Resorption, Med. Welt. **10:** 477 (April) 1936.

[30] Kendall, A. I., and Chinn, Herman: A Method for Isolation of Ascorbic Acid Fermenting Bacteria. Proc. Soc. Exper. Biol. & Med. **38:** 8 (Feb.) 1938; Decomposition of Ascorbic Acid by Certain Bacteria, J. Infect. Dis. **62:** 330 (May-June) 1938.

After absorption vitamin C may be detected in increased quantities in the blood plasma, by which it is transported to the various tissues of the body. Whether a storage of vitamin C occurs in the animal body is still a debatable question.[33] This is indicated by the necessity for a continuous food supply in species incapable of synthesizing vitamin C. Because of the ease with which vitamin C can be determined chemically, many studies have been made concerning its metabolism. Large doses of the vitamin have been administered and measurements made of the amounts excreted in the urine. Johnson and Zilva [34] consider that "saturation" of the tissues with vitamin C has been reached when the ingestion of a certain amount of vitamin C is roughly equaled by the daily excretion of the vitamin in the urine. Succeeding investigators have attempted to determine the saturation of the organism by oral or intravenous administration of test doses of ascorbic acid and subsequent measurement of its excretion in the urine. The majority of investigators employing urinary excretion tests have failed to recognize the possibility that absorp-

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31. Wilder, R. M., and Wilbur, D. L.: Diseases of Metabolism and Nutrition, Arch. Int. Med. **59**: 512 (March) 1937.

32. Hou, H. C.: Comparison of Oral and Subcutaneous Administration of Protective Doses of Ascorbic Acid (Vitamin C), Proc. Soc. Exper. Biol. & Med. **32**: 1391 (June) 1935.

33. Zilva, S. S.: Vitamin C Requirements of the Guinea Pig, Biochem. J. **30**: 1419 (Aug.) 1936.

34. Johnson, S. W., and Zilva, S. S.: The Urinary Excretion of Ascorbic Acid and Dehydroascorbic Acids in Man, Biochem. J. **28**: 1393 (No. 4) 1934.

tion from the intestinal tract may not be complete.[35] Further, they have arbitrarily stated that various amounts ranging from 30 to 75 per cent of the test dose are excreted on reaching "a state of saturation." If subjects on a previous minimal vitamin C diet require a total of from 2,000 to 3,000 mg. of ascorbic acid before appreciable amounts are excreted in the urine, must it be assumed that the total amount of vitamin administered found its way to the tissues? It should be obvious that, before quantitative statements are derived, consideration must be given to questions of destruction in and absorption from the intestinal tract, and excretion by way of the feces.

We have repeatedly observed that, on the oral administration of large quantities of vitamin C, only small amounts are excreted in the urine, while a steady rise in plasma level occurs. If the oral ingestion is continued for a sufficient period, a point of plasma elevation will be attained, following which a rapid urinary excretion of a large part of the ingested ascorbic acid occurs. We believe that the plasma level at which this quantitative urinary excretion occurs shows considerable individual variation.

Everson and Daniels [36] state that ascorbic acid metabolism may be compared with nitrogen metabolism in that the urinary excretion of vitamin C is of both endogenous and exogenous origin. Hawley and others [37] report that the amount of vitamin C excreted in the urine may vary according to the acid-alkali content of the diet, a highly alkaline urine having lower amounts than a highly acid urine. Daniels [38] reports that therapeutic doses of acetylsalicylic acid increase the excretion of vitamin C in the urine of children, while Youmans [39] states that it does not increase or decrease excretion of vitamin C in the urine of adults. It has been reported by Ahmad[40] that diets high in protein and fat cause an increased urinary excretion of vitamin C. On the other hand, Heinemann[41] noted that the total reducing power of the urine varies directly with the protein intake, while the vitamin C excretion remains uninfluenced.

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[35] Storage of Vitamin C, editorial, J. A. M. A. **108**: 395 (Jan. 30) 1937.

[36] Everson, G. J., and Daniels, A. L.: Vitamin C Studies with Children of Preschool Age, Nutrition **12**: 15 (July) 1936.

[37] Hawley, E. E.; Frazer, J. P.; Button, L. L., and Stephens, D. J.: Effect of the Administration of Sodium Bicarbonate and of Ammonium Chloride on the Amount of Ascorbic Acid Found in the Urine, Nutrition **12**: 215 (Aug.) 1936.

[38] Daniels, Amy L., and Everson, Gladys J.: Influence of Acetylsalicylic Acid (Aspirin) on Urinary Excretion of Ascorbic Acid, Proc. Soc. Exper. Biol. & Med. **35**: 20 (Oct.) 1936.

[39] Youmans, J. B.; Corlette, M. B.: Frank, Helen, and Corlette, Mildred: Failure of Acetylsalicylic Acid to Affect Excretion of Ascorbic Acid (Vitamin C) in Urine, Proc. Soc. Exper. Biol. & Med. **36**: 73 (Feb.) 1937.

Diuretic Effect.—Abbasy[42] has noted that, following the administration of a test dose (700 mg. of ascorbic acid to 10 stone, 140 pounds, 63.5 Kg. of body weight), the volume of urine, as well as the excretion of vitamin C in the urine, showed a definite rise. He therefore attributed a diuretic action to the vitamin. As a result of an extended study of the urinary output of nine patients with cardiac disease, Evans[43] states that vitamin C was found to induce a greater diuresis than digitalis but less than theobromine, theobromine with sodium salicylate, and ammonium chloride.

Blood Pressure.—It was noted by Kasahara and Kawamura[44] that guinea pigs showed a rise in blood pressure when injected with 100 mg. per kilogram or greater doses of ascorbic acid and that monkeys showed a rise in blood pressure starting with injections of 40 mg. per kilogram and a greater rise with doses of from 100 to 200 mg. per kilogram. The authors conclude that the blood pressure rises in monkeys and guinea pigs following injections only of large amounts of ascorbic acid.

Capillary Action.—While it has been shown histologically that vitamin C regulates the intercellular substance of the capillary wall, a clear relationship between capillary resistance of the skin and dietary intake of vitamin C has not been definitely established.[45] Göthlin[46] has recently attempted to correlate capillary resistance measured by his positive pressure method with the ascorbic acid content of blood. He found increased capillary fragility in individuals with blood levels of 1 mg. per liter or less. In more than twenty cases of both infantile and adult scurvy observed by us, we have never obtained such low blood values.

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[40] Ahmad, Bashir: Observations on Excretion of Vitamin C in Human Urine, Biochem. J. **30**: 11 (Jan.) 1936.

[41] Heinemann, Martin: On Relation Between Diet and Urinary Output of Thiosulfate and Ascorbic Acid; Human Requirements for Vitamin C, Biochem. J. **30**: 2299 (Dec.) 1936.

[42] Abbasy, M. A.: The Diuretic Action of Vitamin C, Biochem. J. **31**: 339 (Feb.) 1937.

[43] Evans, William: Vitamin C in Heart Failure, Lancet **1**: 308 (Feb. 5) 1938.

[44] Kasahara, M., and Kawamura, R.: Ueber den Einfluss der l-Ascorbinsäure auf den Blutdruck, Klin. Wchnschr. **16**: 1543 (Oct.) 1937.

[45] Abt, Farmer and Epstein.[21] Wilder and Wilbur.[31] Abbasy.[42]

[46] Göthlin, G. F.: When Is Capillary Fragility a Sign of Vitamin C Subnutrition in Man? Lancet **2**: 703 (Sept. 18) 1937; Acta paediat. **20**: 71, 1937.

Effect of Anesthesia.—According to Bowman and Muntwyler,[47] urinary excretion of vitamin C is increased following ether anesthesia in the dog, rat and guinea pig. The ascorbic acid content of the kidneys, liver, adrenals and spleen was found to be lessened after anesthesia. Bersin and his co-workers[48] found that excretion of ascorbic acid was diminished in the urine following ether and chloroform anesthesia in guinea pigs, on administration of a test dose of vitamin C intravenously.

RELATION OF VITAMIN C TO SCURVY

Scurvy is a disease for which vitamin C is the sole therapeutic agent. The specificity of vitamin C in scurvy was demonstrated long before the isolation of the vitamin and, as soon as the vitamin was available as a pure chemical substance, it was successfully used both clinically and experimentally in the treatment of scurvy. According to Finkle,[49] a low body level of vitamin C is etiologic for no other disease than scurvy, with the possible exception of lupus erythematosus. As has been previously stated, the blood plasma levels are extremely low in human scurvy. Abt,[50] in reporting his early studies on human scurvy, showed that the dietary history and clinical signs and symptoms parallel the low blood plasma values and that the plasma content shows a rapid rise in a healing scurvy. With the aid of the micromethod described by us, tolerance tests were made on patients recovering from scurvy, the ascorbic acid content of the plasma being determined hourly and curves plotted. The initial level rises slightly but usually returns to a low level.[17] As the patient continues on a high vitamin C intake, fasting blood levels on successive days attain higher values. Tolerance tests performed at these times by the oral administration of 10 mg. of ascorbic acid per kilogram of body weight show, with fair uniformity, a higher value at the peak of each succeeding curve. The peak is usually reached by the second hour, and the rise (difference between the initial fasting level and the value attained at the peak) increases until a state is attained in which the body promptly excretes the major part of the ingested ascorbic acid.

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[47] Bowman, D. E., and Muntwyler, E.: Further Experiments upon the Excretion of Ascorbic Acid in the Urine Following Ether Anesthesia, J. Biol. Chem. **14:** 114, 1936; Ascorbic Acid Content of Tissue Following Ether Anesthesia, Proc. Soc. Exper. Biol. & Med. **35:** 557 (Jan.) 1937.

[48] Bersin, T.; Lauber, H. J., and Nafziger, H.: Der Einfluss der Narkose und Operation auf den Vitamin C Haushalt, Klin. Wchnschr. **16:** 1272 (Sept. 11) 1937.

[49] Finkle, Philip: Vitamin C Saturation Levels in Body in Normal Subjects and in Various Pathological Conditions, J. Clin. Investigation **16:** 587 (July) 1937.

[50] Abt, A. F.: Newer Knowledge of Vitamin C, Radiol. Rev. & Mississippi Valley M. J. **59:** 106 (May) 1937.

Ascorbic acid may be given intramuscularly or intravenously for the cure of scurvy when nausea, emesis or extreme prostration prevents its oral administration. It is interesting to note that Hess and Unger[51] in 1918 administered neutralized orange juice intravenously to three patients for whom oral administration was impossible.

Jackson and Park[52] have reported a case of congenital scurvy. By determining the vitamin C content of the blood obtained simultaneously from the cord of the infant and the vein of the mother immediately after delivery, Abt, Farmer and Epstein[21] found practically identical values from the two sources. From these observations it may be concluded that the fetus is dependent for its source of vitamin C on the maternal dietary. If the vitamin intake of the mother is extremely low during pregnancy, congenital scurvy may consequently result.

Goettsch[53] has noted in treating infantile scurvy that a single massive dose of vitamin C permits the healing of the scurvy as effectively as the same amount of vitamin in divided doses. Observing calcification of subperiosteal hematomas, she noted that healing commenced as early as the forty-eighth hour after the intravenous injection of the large doses of vitamin. Aub[54] reports that vitamin C is a factor in calcium deposition in the bones. Its addition to a scorbutic diet causes a rapid deposition of calcium at the epiphysial ends of bones and in trabeculae. The effect is probably due to the necessity of adequate amounts of vitamin C for normal production of the organic matrix in which the lime salts are deposited.

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[51] Hess, A. F., and Unger, L. J.: Experiments on Antiscorbutics; Report of an Antiscorbutic for Intravenous Use, Proc. Soc. Exper. Biol. & Med. **15:** 141 (May) 1918.

[52] Jackson, Deborah, and Park, E. A.: Congenital Scurvy: Case Report, J. Pediat. **7:** 741 (Dec.) 1935.

[53] Goettsch, Elvira: Treatment of Infantile Scurvy with Cevitamic Acid, Am. J. Dis. Child. **49:** 1441 (June) 1935.

[54] Aub, J. C.: The Use of Calcium and the Choice of a Calcium Salt, J. A. M. A. **109:** 1276 (Oct. 16) 1937.

Hess[55] has noted the development of scurvy in infants on a diet which contained sufficient vitamin to be ordinarily antiscorbutic. These infants recovered when given larger amounts of vitamin C orally. Hess felt that the development of scurvy in these cases was due to a failure of absorption of the ingested vitamin. Hagmann[56] reported that scurvy developed in a 2½ months old infant receiving 4 drachms (15 cc.) of orange juice daily. Large doses of vitamin C taken orally failed to cure the scurvy, while vitamin C given intravenously caused an effective therapeutic response. Hagmann attributes the failure of the orally administered vitamin to effect a cure to a temporary faulty absorption from the gastrointestinal tract.

It has been noted that the urinary excretion of vitamin C in scurvy is low. Schultzer[57] has recently reported an average daily excretion of 11 mg. in the urine of a scorbutic patient before treatment. After daily intravenous injections of 40 mg. of the crystalline vitamin, the twenty-four hour urinary excretion rose to 16 mg. in the second week and to 26 mg. in the fourth week. Schultzer points out that on the basis of earlier experience the oral administration of 15 mg. of vitamin C daily was considered an adequate protective dose. Since a daily intravenous injection of 40 mg. of the vitamin was found to be an adequate curative dose, he suggests that the daily human requirement may be less than 40 mg. It has been[58] pointed out that, in the guinea pig, parenterally administered vitamin C is approximately twice as effective as a similar dose given orally.

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[55] Hess, A. F.: Infantile Scurvy, in System of Pediatrics (I. A. Abt, Editor), Philadelphia, W. B. Saunders Company, 1923, vol. II, chapter 30, p. 849.

[56] Hagmann, E. A.: Active Scurvy in an Infant Receiving Orange Juice, J. Pediat. **11:** 480 (Oct.) 1937.

[57] Schultzer, P.: Saturation of a Scurvy Patient with Small Doses of Ascorbic Acid, Biochem. J. **31:** 1934 (Nov.) 1937. Abt, A. F., and Farmer, C. J.: Exhibit on Vitamin C, Kansas City Session A. M. A., May 1936.

[58] Hou.[32] Wright, I. S.; Lilienfeld, Alfred, and MacLenathen, Elizabeth: Determination of Vitamin C Saturation, Arch. Int. Med. **60:** 264 (Aug.) 1937.

LATENT SCURVY

The question of a prescorbutic state or latent scurvy was first emphasized by Hess[59] in 1917. It was his contention that this state might be analogous to latent tetany. Hess and succeeding observers attempted to demonstrate this condition by the use of various tests for capillary fragility in the skin. Since the chemical isolation of the vitamin, various observers have attempted, through urinary excretion tests, to demonstrate this condition. As we have previously stated, Zilva,[33] employing the urinary excretion tests in experimental animals, casts doubt on the nature of this latent condition.

In our work we have repeatedly pointed out that fasting blood plasma levels parallel the vitamin C intake. In healthy individuals on what is considered an adequate vitamin C intake the blood plasma value will be 0.7 mg. per hundred cubic centimeters or above. Blood plasma values below 0.7 mg. per hundred cubic centimeters are subnormal or at least suboptimal. Active scurvy may occur with values ranging up to 0.4 to 0.5 mg. per hundred cubic centimeters (chart 2). Most authors reporting on blood plasma determinations are in close agreement with these figures with the exception of Ingalls,[60] whose figures are very low: from 0.15 to 0.30 mg. per hundred cubic centimeters of blood plasma in asymptomatic scurvy and from 0 to 0.15 mg. in active scurvy. The low values obtained by this author may be due to the fact that potassium cyanide was added to the blood, which was in some cases permitted to stand instead of being immediately deproteinized with metaphosphoric acid and titrated with 2:6 dichlorophenolindophenol. It remains for future investigation to prove or disprove the existence of a latent or prescorbutic state. With respect to the development of human scurvy, it should be emphasized that scurvy may surreptitiously develop in patients under treatment for other conditions, if adequate protective vitamin C content of the diet has been overlooked.

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59. Hess, A. F.: Subacute and Latent Infantile Scurvy: The Cardiorespiratory Syndrome (A New Sign), J. A. M. A. **68**: 235 (Jan. 27) 1917.

60. Ingalls, T. H.: Studies on Urinary Excretion and Blood Concentration of Ascorbic Acid in Infantile Scurvy, J. Pediat. **10**: 577 (May) 1937.

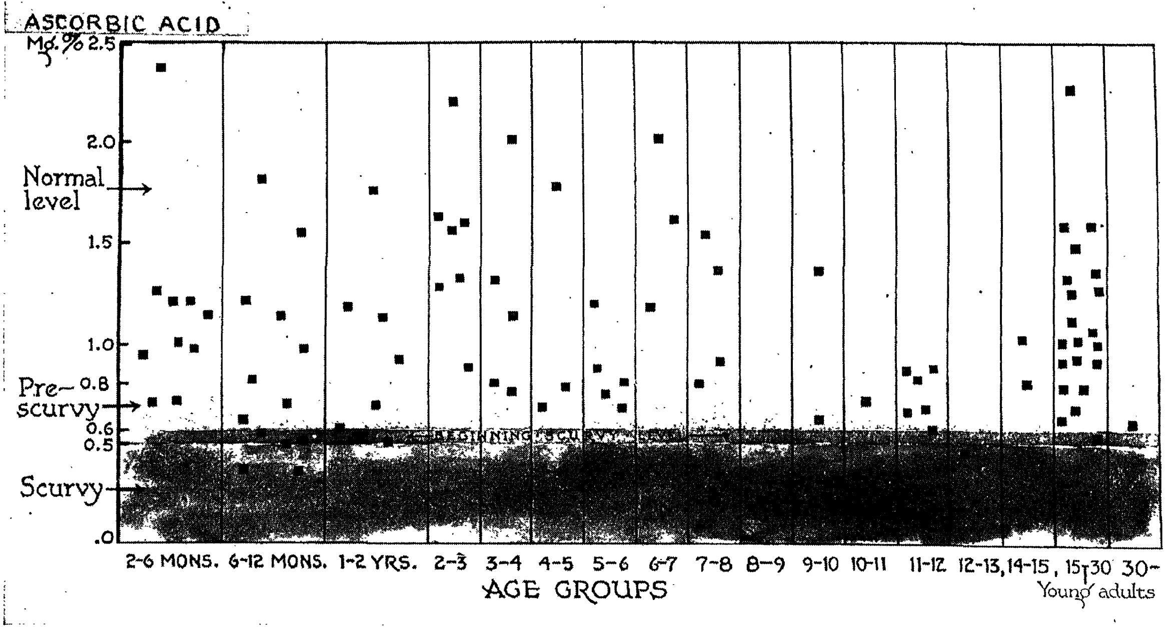

Chart 2.—Range of blood values for different age groups on adequate and subnormal diets, and in patients with scurvy.

ANEMIA AND VITAMIN C

Mettier, Minot and Townsend[61] reported that anemia is commonly found in adults with a vitamin C deficiency. They believe that vitamin C can have a specific effect on erythropoiesis when there has been a chronic lack of this vitamin. Anemia is more frequently associated with scurvy in the adult than in the infant, judging from clinical reports. Alfred Hess[62] noted that anemia was not constantly associated with infantile scurvy; in fact, in some of his scorbutic infants he reported a polycythemia. Rohmer and Bindschedler[63] reviewed the literature, noted the disagreement of the various authors on the association of anemia with infantile scurvy, and concluded that anemia is not an essential symptom of vitamin C deficiency. Hans Aron[64] also reported that anemia is not always associated with scurvy and that when it occurs it is not due to loss of blood, as was formerly believed. He stated also that in many instances it is difficult to determine whether one is dealing with a scurvy accompanied by anemia or an alimentary anemia complicated by scurvy. Parsons and Hawksley[65] suggested that hematopoiesis does not occur in the absence of vitamin C. They believed that in some cases of deficiency anemia more than one factor may be lacking and stated that "a child suffering from nutritional anemia may also develop scurvy, and the anemia of scurvy, in which case for its complete cure vitamin C as well as iron will be required." It is the opinion of Cooley[66] that there is no direct relationship between any of the avitaminoses and a definite type of anemia. Gingold[67] concluded that ascorbic acid has no effect on the normal or pathologic hemopoietic organs and is not, therefore, indicated in the treatment of pernicious anemia, secondary anemias or leukemias.

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[61] Mettier, S. R.; Minot, G. R., and Townsend, W. C.: Scurvy in Adults, Especially Effect of Food Rich in Vitamin C on Blood Formation, J. A. M. A. **95:** 1089 (Oct. 11) 1930.

[62] Hess, A. F.: Scurvy, Past and Present, Philadelphia, J. B. Lippincott Company, 1920.

[63] Rohmer, P., and Bindschedler, J. J.: L'anémie préscorbutique du nourrison, Acta. pediat. **13:** 399 (June) 1932.

[64] Aron, Hans: Die Nahroschaden des Kindes, Berlin and Vienna, Urban und Schwarzenberg, 1928, p. 103.

[65] Parsons, L. G., and Hawksley, J. C.: Studies in Anemias of Infancy and Early Childhood; Anhematopoietic Anemias (Deficiency Diseases of Erythron); Nutritional Anemia and Anemias of Prematurity, Scurvy and Celiac Disease, Arch. Dis. Childhood **8:** 117 (April) 1933.

[66] Cooley, Thomas B.: Practice of Pediatrics, Joseph Brennemann, Editor, Hagerstown, Md., W. F. Prior Company, 1937, vol. III, chapter 16, p. 9.

[67] Gingold, N.: The Action of Ascorbic Acid (Vitamin C) on the Hemopoietic Apparatus Under Normal and Pathologic Conditions (Exclusive of Hemorrhagic States), Sang **11:** 392, 1937.

We have recently noted a 10 months old infant suffering with scurvy who was being fed on a diet containing a mixture of numerous proprietary products, so that the diet was high in calcium, iron and vitamins A, B_1 and D; the diet lacked only vitamin C. The infant was of a ruddy complexion, the red blood count was 4,800,000 and the hemoglobin 75 per cent (Sahli). The conditions in the case corresponded to our experimental experience with young guinea pigs, namely that anemia does not develop either clinically or experimentally when an adequate supply of iron is present in the scorbutic diet. When anemia accompanies lack of vitamin C it is probably due to a generally deficient diet in which substances other than vitamin C, especially iron, have been lacking. An accompanying infection may be a causative factor in the production of such anemia. While it is felt by some that the anemia of vitamin C deficiency may be one of commission, the result of a dysfunctioning of the red blood cell-forming elements, it is probably more often a condition produced by omission, namely of iron, in the generally deficient diet.

Heilmeyer [68] injected iron ascorbate intravenously in patients suffering from secondary anemia and noted good results, especially in those patients in whom oral administration of iron was interfered with by metabolic disturbances. He further administered iron ascorbate orally and noted a better absorption with this preparation than with other iron products. He was able to obtain a full iron effect with doses of from 300 to 600 mg. daily. Glanzmann [69] has recommended iron ascorbate in the treatment of infantile anemia of the alimentary type. Pijoan [70] later made a similar report. It is evident that the need of a preparation such as iron ascorbate is limited.

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68. Heilmeyer, H.: Die Behandlung eisen emfindlicher Anämien mit Askorbinsauren Eisen, Deutsches Arch. f. klin. med. **179**: 216 (Sept.) 1936.

69. Glanzmann, E.: Zur Behandlung der Kinderänamien mit ascorbinsauren Eisen, Schweiz. Med. Wchnschr. **67**: 436 (May 15) 1937.

70. Pijoan, M.: Antiscorbutic Properties of a Salt of Iron and Ascorbic Acid, Science **86**: 80 (July 23) 1937.

LEUKEMIA AND VITAMIN C

Stephens and Hawley[71] have noted that the vitamin C content of leukocytes is considerably greater than that of the plasma or erythrocytes. They noted an extremely high value for the ascorbic acid of the whole blood in leukemias. Eufinger and Gaehtgens[72] have reported the treatment of a case of myeloid leukemia in which the white count was brought back to normal by the injection of 2,000 mg. of ascorbic acid.

RELATION OF VITAMIN C TO HEMORRHAGIC DISEASES

Following the isolation of vitamin C in crystalline form there was a great impetus for investigations of its therapeutic value in hemorrhagic diseases and hemorrhage in other diseases. At first, numerous reports appeared claiming good results from the use of vitamin C in symptomatic purpura, thrombopenic purpura, Schönlein's and Henoch's purpuras, and hemophilia. Favorable claims also were made for such conditions as leukemia, acute hemorrhagic nephritis, metrorrhagia, pulmonary and gastric hemorrhages, and intestinal hemorrhage in ulcerative colitis and following perforations in typhoid. These were usually accounts of success in isolated cases.[73] It soon became evident that these early enthusiastic claims could not be substantiated.

In examining several patients with thrombopenic purpura whose blood components had been studied for a considerable time prior to the therapeutic attempts with vitamin C, we were able, with Dr. Mila Pierce,[74] to demonstrate no appreciable improvement in the thrombopenia. The capillary resistance of the skin remained abnormally low after the administration of large doses of vitamin C both orally and intravenously.

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[71] Stephens, D. J., and Hawley, E. E.: Partition of Reduced Ascorbic Acid in Blood, J. Biol. Chem. **115:** 653 (Oct.) 1936.

[72] Eufinger, H., and Gaehtgens, G.: Ueber die Einwirkung des Vitamin C auf das Pathologisch veränderte weisse Blutbild, Klin. Wchnschr. **15:** 150 (Feb. 1) 1936.

[73] Boger, A., and Schröder, H.: Ueber die Stillung schwerster Blutungen bei allen Formen der hemorrhagischen Diathese und der Hemophilie durch parenterale Zufuhr von C-Vitamin, München. med. Wchnschr. **81:** 1335 (Aug. 24) 1934. Vogt, E.: Ueber die Behandlung gynäkologischer Blutunger mit Vitamin C, ibid. **82:** 263 (Feb. 14) 1935. Engelkes, N.: Treatment of Hemorrhagic Disorders with Vitamin C, Lancet **2:** 1285 (Dec. 7) 1935.

[74] Abt, A. F.: Physiology of Ascorbic Acid in Normal and Abnormal States, J. Physiol. U. S. S. R. **22:** 807 (July) 1937.

Several investigators[75] in this country have similarly been unable to report beneficial effects after treatment with vitamin C in cases of true thrombopenic purpura and hemophilia.

In a recent review on the hemorrhagic states, Witts[76] corroborated the negative therapeutic effect of vitamin C in nonscorbutic hemorrhagic states. This author mentioned the possibility of scurvy being associated with a hemorrhagic disease, the disappearance of some of the symptoms following treatment with the vitamin being due to its effect on the associated scurvy. An interesting report by Neuweiler[77] concerns the vitamin C content of the blood serum of women during normal menstruation. In this study the author found that the vitamin C content of the blood serum did not vary during the menstrual periods of three normal women. Szent-Györgyi and others,[78] in their early work with impure preparations of vitamin C, believed that successful therapeutic effects were attained in some of the nonscorbutic hemorrhagic diseases. He and his co-workers subsequently isolated a flavin which they term "vitamin P" and which they believed was responsible for the effect. In a recent report they have stated that this substance requires for its activity the presence of traces of ascorbic acid and, in the entire absence of ascorbic acid, vitamin P is inactive. According to these results it may be concluded that pure vitamin C has a negative therapeutic action on hemorrhagic diseases of a nonscorbutic etiology. The evidence for the existence of vitamin P is doubtful, however, and it is clear that further work will have to be done in this field before conclusions can be reached.

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75. Wright, I. S., and Lilienfeld, Alfred: Pharmacologic and Therapeutic Properties of Crystalline Vitamin C (Cevitamic Acid) with Especial Reference to Its Effects on Capillary Fragility, Arch. Int. Med. **57:** 241 (Feb.) 1936. Stephens, D. J., and Hawley, E. E.: Relationship of Vitamin C to the Hemorrhagic Diatheses, J. Lab. & Clin. Med. **22:** 173 (Nov.) 1936. Finkle, Philip: Vitamin C Saturation Levels in Body in Normal Subjects and in Various Pathological Conditions, J. Clin. Investigation **16:** 587 (July) 1937.

76. Witts, L. J.: The Hemorrhagic States, Brit. M. J. **2:** 689 (Oct. 9) 1937.

77. Neuweiler, W.: Vitamin C-Spiegel in Blute und Menstruation, Klin. Wchnschr. **16:** 926 (Sept. 4) 1937.

78. Szent-Györgyi, A.; Armento, S.; Bentsath, A.; Beres, T., and Rusznyak, S.: Ueber den Einfluss von Substanzen der Flavongruppe auf die Permeabilität der Kapillaren, Vitamin P, Deutsche med. Wchnschr. **62:** 1325 (Aug. 14) 1936.

RELATION OF VITAMIN C TO OTHER CONDITIONS

Teeth.—Because gingivitis and hemorrhage into the gums constitute such a striking symptom of scurvy, the claim that vitamin C has a direct relationship with the well-being of the teeth has been made for a long period. Hanke,[79] in a study on children, concluded that the addition of 1 pint of orange juice and the juice of one lemon to a diet lacking in vitamin C, but otherwise normal, led in many cases to disappearance of gingivitis and an arrest or a reduction of the intensity of dental caries. Keeton[80] has administered 400 mg. of vitamin C daily for four or five days prior to the extraction of a tooth and reported that the effect on healing is striking.

Most of the evidence that vitamin C is related to normal development of the teeth has been deduced largely from experimental work on the guinea pig. The incisor teeth of the guinea pig differ from those of man in that they are constantly growing. One is led to question, therefore, the permissibility of carrying over to man, without reservation, all experimental evidence on this point.

Fish and Harris[81] in their recent work on guinea pigs hold untenable the view of the earlier writers, who noted the formation of "pulp bones" in partial vitamin C deficiency and regarded this occurrence as a type of neoplastic change. By use of a technic in which a series of deficient and normal diets were fed, they noted that vitamin C is necessary for promoting the functional activity of formative cells such as the odontoblasts, ameloblasts and osteoblasts. They noted that in mild chronic vitamin C deficiency the "pulp bone" is the result of a partial degeneration of the odontoblasts, forming a scar tissue.

Wolbach[11] has pointed out that in the growing teeth of guinea pigs a lack of vitamin C causes the formation of dentin to cease and the pulp to become separated from the dentin by a liquid presumably produced by the odontoblasts. In the healing process in guinea pigs the formation of dentin is resumed. Wolbach concluded that, in infants, teeth in the process of formation may possibly be affected by scurvy in a manner similar to that noted in the guinea pig, but that specific evidence of this in the human being is lacking. Hou[82] has reported similar observations in the scorbutic guinea pig. As evidence of a change in mineral content, Toverud[83] has shown that the incisor teeth of scorbutic guinea pigs contain less ash than those of normal animals. Furthermore, the ash is lower in calcium and higher in magnesium that that from teeth of normal controls. The older writers recognize the loosening of teeth in the scurvy of both man and animals, and Boyle, Bessey and Wolbach[84] more recently have pointed out that, besides striking alterations in tooth pulp and dentin, there may be changes in the peridental soft and calcified tissues. They concluded that there may be two types of pyorrhea, a local inflammatory disease and a systemic process causing diffuse atrophy of the alveolar bone. This systemic type of pyorrhea, as it occurs in infantile scurvy, is similar to that found in guinea pigs on a vitamin C-deficient diet. In a limited number of patients with this type of pyorrhea, they have noted a correlation between low blood ascorbic acid values and rarefaction of the alveolar bone. They believe that a low vitamin C intake is an important factor in the production of this type of systemic pyorrhea. The authors made no statement as to the therapeutic effect which vitamin C might have on this type of pyorrhea. It is probable that the beneficial effects noted by Hanke after the addition of large amounts of orange juice were due to amelioration of this systemic type of pyorrhea known to be caused by deficiency of the vitamin. Although there is still a dearth of exact knowledge of vitamin C in its relation to dental and gingival disease in man, there is a general unanimity of opinion that an adequate intake of vitamin C is necessary for normal tooth growth and tooth structure, and the maintenance of healthy gums in man.

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[79] Hanke, Milton H.: Diet and Dental Health, University of Chicago Press, 1933, p. 143.

[80] Keeton, R. W.: Relation of Metabolic Diseases to Dentistry, J. Am. Dent. A. **24:** 1336 (Aug.) 1937.

[81] Fish, E. W., and Harris, L. J.: The Effects of Vitamin C Deficiency on Tooth Structure in Guinea Pigs, Phil. Tr. Roy. Soc. London, 223B, 1933, p. 489.

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[82] Hou, H. C.: A Comparative Study of the Vitamin C Content of Several Varieties of Chinese Oranges, Chinese J. Physiol. **9**: 223 (Aug. 15) 1935.

[83] Toverud, G.: The Influence of Diet on Teeth and Bone, J. Biol. Chem. **58**: 358 (Dec.) 1923.

[84] Boyle, P. E.; Bessey, O. A., and Wolbach, S. B.: Experimental Alveolar Bone Atrophy Produced by Ascorbic Acid Deficiency and Its Relation to Pyorrhea Alveolaris, Proc. Soc. Exper. Biol. & Med. **36**: 733 (June) 1937.

Eye.—Whether deficiency of vitamin C has any significance in the etiology of cataract has been a matter of dispute. Various workers have found that, as in other tissues of the body, the concentration of vitamin C in the crystalline lens of scorbutic guinea pigs is lower than normal.[85] Low values have been reported in the cataractous lens.[86] Some investigators have been able to produce cataract in some guinea pigs by feeding them a scurvy-producing diet,[87] especially if the aqueous humor was drained by paracentesis, but others have not been able to repeat the experiment.[88] Vitamin C is present in the normal lens and vitamin C depletion might conceivably predispose the lens to a greater degree of susceptibility to agents causing cataract which ordinarily do not affect the normal eye. Bellows [89] has studied the level of vitamin C in the blood of cataractous patients and found it to be definitely lower on the average than that of noncataractous persons of similar social status and age group (from 60 to 80 years).

Skin.—Vitamin C apparently has a definite relation to pigmentation of the skin. A lessened pigmentation in patients suffering with Addison's disease has been observed [90] after vitamin C had been administered for some time. Schroeder and Einhauser [91] administered 300 mg. of vitamin C intravenously for fourteen days and noticed a decreased pigmentation of the face in a case of pernicious anemia. Cornbleet [92] noted that the excess pigment in the skin in Addison's disease and in scurvy is absorbed when vitamin C is administered.

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[85] Von Euler, H., and Malmberg, M.: Ueber die Antiskorbutische Wirkung der Augenlinsen und über ihren Gehalt an Reduktonen und Sulfhydrylen, Ztschr. f. physiol. Chem. **230:** 225 (Oct.) 1934. Johnson.[88]

[86] Von Euler, H., and Martius C.: Ueber den Gehalt der Augenlinsen und Sulfhydrylverbindungen und an Ascorbinsäure, Ztschr. f. physiol. Chem. **222:** 65 (Oct.) 1933.

[87] Monjukowa, N. K., and Frandkin, M. J.: Neue experimentelle Befunde über die Pathogenese der Katarakt, Arch. f. Ophth. **133:** 378, 1935.

[88] Johnson, S. W.: Cataract and Ascorbic Acid in Guinea Pig Eye, Biochem. J. **30:** 1430 (Aug.) 1936.

[89] Bellows, J. G.: Biochemistry of Lens; Cevitamic Acid Content of Blood and Urine of Subjects with Senile Cataract, Arch. Ophth. **15:** 78 (Jan.) 1936.

[90] Wilkinson, J. F., and Ashford, C. A.: Vitamin C Deficiency in Addison's Disease, Lancet **2:** 967 (Oct. 24) 1936. Hoff.[103]

[91] Schroeder, H., and Einhauser, M.: Ueber einen Zusammenhang zwischen gestorter Vitamin-C-Resorption und pathologischer Pigmentierung bei Gastroenteritis und Achyliagastrica. München. med. Wchnschr. **83:** 923 (June 5) 1936.

[92] Cornbleet, Theodore: Vitamin C and Pigment, Arch. Dermat. & Syph. **35:** 471 (March) 1937.

Volpe[93] treated several patients suffering with psoriasis with large doses of ascorbic acid and noted striking improvement. We ourselves have noted depigmentation of the skin following massive vitamin C therapy in Addison's disease. In the case of a Negro, a daily oral dose of 450 mg. over a period of several months produced a noticeable mottling depigmentation below the eyes. It should be noted that large doses, far beyond the normal requirement, have been used to produce these therapeutic results.

The suggestion has been made[49] that vitamin C may be specific in lupus erythematosus as well as in scurvy.

ENDOCRINE DISTURBANCES

Several observers have pointed out the possibility of a relationship between ascorbic acid and thyroid gland function. Demole and Ippen[94] have demonstrated that loss of weight in guinea pigs on a scorbutic diet could be checked by the administration of 0.5 mg. of ascorbic acid daily. If, however, the animals received 0.1 mg. of thyroxin subcutaneously, the weight loss continued until the amount of daily oral ascorbic acid administration had been raised to from 10 to 20 mg. Demole and Ippen interpreted this as evidence of the antithyrotoxic action of ascorbic acid. Heyl[95] has reported a morphologic change in the thyroid gland induced by ascorbic acid similar in appearance to that obtained by the injection of the thyrotropic hormone of the anterior pituitary lobe. If the extract of the anterior pituitary is subjected to oxidative processes, shown by control experiment to be severe enough to destroy ascorbic acid, the pituitary extract still remained active. The author concluded that, in the case of the thyrotropic action of the anterior pituitary, a substance other than vitamin C is concerned. Spence and Scowen[96] have shown that the daily administration of large doses of ascorbic acid does not prevent the occurrence of thyroid hyperplasia in guinea pigs receiving injections of extract of anterior pituitary containing thyrotropic hormone. Marine and his associates [97] have noted the protective action of ascorbic acid against hypertrophy of the thyroid gland in guinea pigs injected with the thyrotropic factor. Sure and Theis [98] indicate that in experimental hyperthyroidism produced in rats by feeding toxic doses of thyroxin a vitamin C disturbance occurred as evidenced by the reduction of the vitamin C content of the adrenal, the thymus and the kidney, similar to that encountered in scurvy in the guinea pig. According to Paal and Brecht,[99] the administration of thyroxin and thyrotropic hormone to guinea pigs and rats leads to a rise in the ascorbic acid content of the adrenal gland and liver. When cortical extract free from ascorbic acid was given together with these substances, no such rise occurred. These controversial reports indicate the need for further experimental work on the relationship between vitamin C and the thyroid gland.

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[93] Volpe, I.: Ueber mehrer Erfolge in der Psoriasis Behandlung mit Vitamin C, Schweiz. med. Wchnschr. **67**: 498 (May 29) 1937.

[94] Demole, V., and Ippen, F.: Die Antithyreotoxische Wirkung von Ascorbinsäure, Ztschr. f. physiol. Chem. **235**: 226, 1935.

[95] Heyl, J. G.: Thyreotroper Effekt von Vitamin C (Ascorbinsäure) und Unabhängigkeit der thyreotropen Wirkung des Hypophysen vorderlappens hiervon, Acta brev. Neerland. **4**: 12, 1934.

[96] Spence, A. W., and Scowen, E. F.: The Effect of Ascorbic Acid on Experimental Goiter, Biochem. J. **29**: 562 (Jan. 15) 1935.

The relationship of vitamin C to dextrose tolerance in the guinea pig was studied by Sigel and King;[100] successive stages of vitamin C depletion induced a corresponding rise in the fasting blood sugar level and a distinctly lowered dextrose tolerance. Pfleger and Scholl [101] have noted that in diabetic patients saturation with vitamin C intensifies the action of insulin, so that the carbohydrate metabolism of the patient could be regulated with smaller doses of insulin. They also noted that increased vitamin C ingestion lessened fatigue in diabetic patients.

The relationship between vitamin C and Addison's disease has been studied. As shown by Harris and Ray,[102] both the cortex and the medulla of the normal adrenal gland have a high ascorbic acid content. In three cases of Addison's disease studied by Wilkinson and Ashford,[90] using the urinary test of Harris and Ray, there appeared to be a vitamin C subnutrition. Wilkinson's patients were being simultaneously treated with small doses of adrenal cortex extract and vitamin C. On this combined therapy a decrease in pigmentation of the skin was noted in two cases three months after discharge. Hoff [103] has also noted a decrease in pathologic pigmentation in Addison's disease.

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[97] Marine, David; Baumann, E. J., and Rosen, S. H.: Effect of Ascorbic Acid on Thyroid and Suprarenals of Guinea Pigs, Proc. Soc. Exper. Biol. & Med. **31:** 870 (April) 1934.

[98] Sure, Barnett, and Theis, R. M.: Hyperthyroidism and Vitamin C, Proc. Soc. Exper. Biol. & Med. **37:** 646 (Jan.) 1938.

[99] Paal, H., and Brecht, K.: Ascorbinsäure und Schilddrusenfunktion, Klin. Wchnschr. **16:** 261 (Feb. 20) 1937.

[100] Sigel, A., and King, C. G.: Relationship of Vitamin C to Glucose Tolerance in Guinea Pig, J. Biol. Chem. **116:** 489 (Dec.) 1936.

[101] Pfleger, R., and Scholl, F.: Diabetes and Vitamin C, Wien. Arch. f. inn. Med. **31:** 169 (Oct. 31) 1937.

[102] Harris, L. J., and Ray, S. N.: Vitamin C in the Suprarenal Medulla, Biochem. J. **27:** 2006 (No. 6) 1933.

In two patients with Addison's disease brought to our laboratory by Dr. Carl Johnson, daily oral administration of 450 mg. of ascorbic acid alone caused considerable depigmentation after from six to ten weeks but failed to improve other addisonian symptoms. This is in agreement with the observation of Siwe,[104] who also noted a decreased pigmentation without relief from the characteristic symptoms of Addison's disease.

GASTROINTESTINAL DISEASES

We have already referred to the subject of absorption, destruction and elimination of vitamin C in the gastrointestinal tract. Alt, Chinn and Farmer [105] found low blood values for vitamin C in patients suffering with achlorhydria. Einhauser [106] has reported an altered absorption of vitamin C in patients suffering with catarrhal gastro-enteritis. Although Stepp [107] points out that gastrointestinal disturbances can be the cause as well as the result of lack of vitamins, it is debatable whether a lack of vitamin C can be looked on as a direct contributing factor to gastric ulcer. In Schultzer's [108] studies the improbability of C-avitaminosis as an etiologic factor in peptic ulcer is pointed out. Harris, Abbasy, Yudkin and Kelly [109] and Archer and

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[103] Hoff, F.: Beiträge zum Problem der krankhaften Hauptpigmentierungen. Deutsche med. Wchnschr. **62:** 129 (Jan. 24) 1936.

[104] Siwe, S.: Das Verhalten der C-Vitamin bei Morbus Addisoni, Klin. Wchnschr. **14:** 1311 (Sept. 14) 1935.

[105] Alt, H. L.; Chinn, Herman, and Farmer, C. J.: The Blood Cevitamic Acid in Patients with Achlorhydria, read before the Central Society for Clinical Research, Chicago, Nov. 5, 1937.

[106] Einhauser, M.: C-Vitamin und Gastroenteritis, Ztschr. f. d. ges. exper. Med. **98:** 461, 1936.

[107] Stepp, W.: Neure Gesichtspunkte in der klinischen Anwendung der rein dargstellten Vitamine, Angewandte Chemie **50:** 30 (Jan. 2) 1937.

[108] Schultzer, P.: Studies on Capillary Resistance: Improbability of C-Vitaminosis as Etiologic Factor in Peptic Ulcer, Acta med. Scandinav. **83:** 555, 1934.

[109] Harris, L. J.; Abbasy, M. A.; Yudkin, John, and Kelly, Simon: Vitamins in Human Nutrition: Vitamin C Reserves of Subjects of Voluntary Hospital Class, Lancet **1:** 1488 (June 27) 1936.

Graham[110] reported low urinary excretion values for patients suffering with gastric ulcer. However, these low excretion values may have been related to the low dietary content of the diets prescribed for ulcer. Several investigators[111] have recently pointed out that the usual diets given patients suffering with peptic ulcer are likely to be deficient in vitamin C. The exact relationship of this dietary deficiency to hemorrhage from the ulcers is as yet unproved, although it would certainly seem advisable to supplement peptic ulcer diets with vitamin C. Portnoy and Wilkinson,[112] using urinary excretion, blood plasma level and intradermal tests, have shown a marked vitamin C deficiency in patients suffering from peptic ulcer. The severest degree of deficiency was found in patients with hematemesis. We ourselves have noted a mild scurvy develop in several patients on strict ulcer diets, because of a lack of vitamin C in the dietary.

Gaehtgens[113] has reported on hypovitaminoses of intestinal origin in pregnancy. He points out that there is an increased vitamin C requirement in pregnancy as the fetus gains its supply of ascorbic acid directly from the food intake of the mother. Gaehtgens reports the case of a pregnant woman who, because of a severe ulcerative colitis and a restricted diet, acquired a severe deficiency of vitamin C, and he points out that, whenever gastrointestinal disturbances develop during pregnancy, it may be necessary to supply the maternal organisms with adequate amounts of vitamin C administered parenterally.

RELATION OF VITAMIN C TO IMMUNITY

A considerable amount of experimental work has been published with regard to the relationship of vitamin C to immunity, and this material has recently been comprehensively reviewed.[114] It is not within the scope of this article to take up in detail all the reports covering this subject. Among the first observers to note an altered immunologic response when ascorbic acid was administered in sufficient doses were Sulzberger and Oser.[115] These authors in a controlled experiment noted that large doses of ascorbic acid reduced and inhibited the susceptibility of the skin of the guinea pig to experimental sensitization with neoarsphenamine. Cormia[116] noted that a high vitamin C diet should be of value to all patients receiving arsphenamine and that intensive therapy with vitamin C may be helpful in treating patients with post-arsphenamine dermatitis. Landfisch[117] similarly reported that he was able to treat twenty-five patients who had previously shown definite sensitization to neoarsphenamine and marked symptoms of intolerance when the arsenical was administered alone by combining ascorbic acid injections with neoarsphenamine or by administering the ascorbic acid one-half hour before the neoarsphenamine. Further evidence of the increased tolerance of the organism for arsphenamine after ascorbic acid therapy has been reported recently by Dainow.[118] Vitamin C is apparently of value in controlling the intolerance to arsenicals which must be intravenously administered.

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[110] Archer, H. E., and Graham, George: The Subscurvy State in Relation to Gastric and Duodenal Ulcer, Lancet **2:** 364 (Aug. 15) 1936.

[111] Rivers, A. B., and Carlson, L. A.: Vitamin C as Supplement in Therapy of Peptic Ulcer: Preliminary Report, Proc. Staff Meet., Mayo Clin. **12:** 383 (June 16) 1937. Eusterman, G. B.: Peptic Ulcer; Medical Management, Minnesota Med. **20:** 766 (Dec.) 1937. Ingalls, T. H., and Warren, H. A.: A Symptomatic Scurvy, New England J. Med. **217:** 443 (Sept. 9) 1937.

[112] Portnoy, Benjamin, and Wilkinson, J. F.: Intradermal Test for Vitamin C Deficiency, Brit. M. J. **1:** 328 (Feb. 12) 1938.

[113] Gaehtgens, G.: Die Beziehungen intestinal bedingter Hypovitaminosen zur Schwangerschaft, Klin. Wchnschr. **16:** 444 (March 27) 1937.

Meyer[119] believes that the toxic symptoms produced by chronic benzene poisoning may be due to an avitaminosis C. Experiments with guinea pigs showed that the toxic effect of an injection of 0.25 cc. of benzene four times a week was counteracted when a daily dose of 20 mg. of ascorbic acid was added to a scorbutic basal diet. As a possible mechanism for this effect, Vauthey[120] has recently suggested that ascorbic acid exerts a stimulatory effect on the liver, resulting in reduced toxicity of the chemical medicament.

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[114] Robinson, E. C.: The Vitamins and Resistance to Infection, Medicine **13:** 123 (May) 1934. Perla, David, and Marmorston, Jessie: Role of Vitamin C in Resistance, Arch. Path. **23:** 543 (April) 1937; Role of Vitamin C in Resistance, ibid. **23:** 683 (May) 1937.

[115] Sulzberger, M. B., and Oser, B. L.: Influence of Ascorbic Acid of Diet on Sensitization of Guinea Pigs to Arsphenamine, Proc. Soc. Exper. Biol. & Med. **32:** 716 (Feb.) 1935.

[116] Cormia, F. E.: Experimental Arsphenamine Dermatitis; Influence of Vitamin C in Production of Arsphenamine Sensitiveness, Canad. M. A. J. **36:** 392 (April) 1937.

[117] Landfisch, S.: Synthetic Ascorbic Acid New Auxiliary Remedy in Arsphenamine Therapy, Polska gaz. lek. **16:** 575 (July 18) 1937.

[118] Dainow, I.: Intolérance aux arsénobenzenes et vitamin C, Presse méd. **45:** 1670 (Nov. 24) 1937.

[119] Meyer, A.: Chronische Benzolvergiftung und Vitamin C, Ztschr. f. Vitaminforsch. **6:** 83 (Jan.) 1937.

Epstein[121] noted that large doses of vitamin C aided in the desensitization of patients suffering with bronchial asthma. Jusatz,[122] using rabbits on a deficiency diet, was able to augment the specific precipitant titer from ten to a hundred fold by adding ascorbic acid to each dose of horse protein intravenously injected. The oral administration of vitamin C failed to increase the specific precipitant titer. Madison and Manwaring[123] have confirmed Jusatz's experiments in rabbits using horse serum plus 100 mg. of crystalline vitamin C. These authors also noted that, when the horse serum and ascorbic acid were injected separately, either at different times into the same vein or into different veins, the antibody stimulation was less pronounced than the effect obtained by mixing the horse serum and vitamin C before injection. Lemke[124] has reported that vitamin C, when injected intravenously into hypersensitive guinea pigs, desensitizes them. However, van Niekerk[125] states that vitamin C has no protective action for guinea pigs against anaphylactic shock to horse serum. He noted that no protective action resulted when the second dose of horse serum was combined with from 25 to 100 mg. of ascorbic acid. Hochwald[126] states that, if 100 mg. of vitamin C is injected into sensitized guinea pigs a few minutes before the shocking dose, no anaphylaxis develops. Solomonica[127] observed that, if his guinea pigs were injected with vitamin C prior to or during the sensitizing period, many of them survived. The protective effects of ascorbic acid were

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120. Vauthey, M.: Vitamin C (1-Ascorbic Acid) and Tolerance for Chemical Medicaments, Chem. Abstr. **32:** 995 (Feb. 10) 1938.

121. Epstein. Alexander: Vitamin C in Desensitization; Advances in Vitamin Treatment, Schweiz. med. Wchnschr. **66:** 1087 (Nov. 7) 1936.

122. Jusatz. H. J.: Der Einfluss der Vitamine auf den Immunitätszustand des tierischen Organismus; wasserlösische Vitamine, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88:** 483 (Aug. 4) 1936.

123. Madison. R. R., and Manwaring, M. H.: Ascorbic Acid Stimulation of Specific Antibody Production, Proc. Soc. Exper. Biol. & Med. **37:** 402 (Nov.) 1937.

124. Lemke, H.: Beeinflussung des anaphylaktischen Shocks der Meerschweinchen durch C-Vitamin, Monatschr. f. Kinderh. **67:** 244 (Nov.) 1936.

125. van Niekerk, J.: Anaphylaxis and Vitamin C, J. Allergy **8:** 446 (July) 1937.

126. Hochwald, A.: Allergiefragen und vitamin C, Zentralbl. f. inn. Med. **56:** 769 (Sept. 21) 1935.

127. Solomonica, Bruno: Vitamin C and Anaphylactic Shock in Guinea Pigs, J. Immunol. **31:** 209 (Sept.) 1936.

much lessened if the vitamin was given later, e. g. shortly before the shocking dose.

Vitamin C has recently been related to the complement content of the blood.[128] Ecker[129] and his co-workers have shown a definite correlation between the complement titer of guinea pig serum and the vitamin C content of the blood serum. When the animals were placed on a scorbutic diet the complement titer dropped, paralleling the decrease in ascorbic acid blood values. The values rose when vitamin C was fed to the scorbutic animals.

Jungeblut[130] noted that vitamin C, when added to tetanus toxin in vitro, inactivates the toxin though it fails to confer complete protection against tetanus intoxication in vivo. This author furthermore states that, as far as its in vitro action is concerned, vitamin C cannot be regarded as a specific detoxicant for any given virus or toxin.

It therefore seems probable that vitamin C, particularly when administered intravenously, is capable of protecting the body against certain drug intolerances, as noted for the arsenicals, of stimulating experimental antibody production, and perhaps of protecting against experimental anaphylaxis. The failure of oral administration is interesting and requires further experimental work for its explanation. The whole field of the relationship of vitamin C to natural resistance and immunity is but in its infancy, and much more experimental and clinical evidence must be accumulated before any definite therapeutic claims seem justifiable.

INFECTIONS AND INFECTIOUS DISEASES

Recent reviews suggest that a low vitamin C intake may be one of the causes of lowering natural resistance. There is evidence also that in the course of certain infections there is a greater demand for vitamin C.[131] There have been numerous suggestive reports of the possible role of vitamin C in infectious diseases but the evidence is not yet clearcut. The effect of vitamin C on the integrity of the tissues is probably concerned with its role in the production of normal intercellular cement substances. This in turn may be prerequisite to the maintenance of natural resistance of the organism to infection.

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[128] Vitamin C and Complement, editorial, J. A. M. A. **110:** 2157 (June 25) 1938.

[129] Ecker, E. E.; Pillemer, L.; Wertheimer, D., and Gradis, H.: Ascorbic Acid and Complement Function, J. Immunol. **34:** 19 (Jan.) 1938.

[130] Jungeblut, C. W.: Inactivation of Tetanus Toxin by Crystalline Vitamin C (l-Ascorbic Acid), J. Immunol. **33:** 203 (Sept.) 1937.

[131] The clinical reports have been most numerous. The reader is referred to the extensive bibliographies to be found in the review by Robinson[114] and the reviews by Perla and Marmorston.[114]

Evidence showing the desirability of trying the effect of administering vitamin C, along with other treatment of infections, has been obtained chiefly by studying the vitamin C content of the blood plasma and the amount of vitamin C excreted in the urine. Low values for either or both have been observed in mild coryzal fever, acute pharyngitis, persistent chronic nasal sinusitis, chronic otitis media, acute respiratory infection, active osteomyelitis, rheumatoid arthritis, and rheumatic fever in children.

The administration of vitamin C has been tested in a number of disease conditions, and favorable reports have appeared in pneumonia, pertussis and rheumatic fever. Negative results have been reported in poliomyelitis, and the results are inconclusive in diphtheria. It is evident that vitamin C is not a specific therapeutic agent in the treatment of any of these diseases. It should not be forgotten, however, that the importance of an ample diet has long been recommended in the treatment of febrile conditions, and such patients have perhaps an increased requirement for all of the vitamins.

WOUND HEALING AND SURGICAL CONDITIONS

Hans Aron [64] noted that in scorbutic children wounds of the skin often remained open without a tendency to heal. Following therapy with vitamin C, he observed that these wounds healed quickly. In a 6 weeks old infant whose laparotomy wound opened postoperatively, Lanman and Ingalls [132] noted a definite early scurvy at autopsy. The authors then performed laparotomy on guinea pigs which had been on previous scorbutic diets. At intervals after operation the animals were killed and the authors report that the wounds broke down more easily in the scorbutic pigs than in the nonscorbutic controls. On histologic examination they noted defective repair of the corium and a poor production of collagen in the scars of the scorbutic animals. This is in agreement with the role which vitamin C plays in the formation of collagen. Taffel and Harvey[133] have recently confirmed the work of Lanman and Ingalls and expressed the opinion that vitamin C deficiency in man may indeed be one of the major causative factors in these cases of wound disruption where there is no evidence of infection of the wound. From the evidence presented one may conclude that vitamin C has an important role in the healing of surgical wounds. When a history of low vitamin C intake or a low plasma value is obtained preoperatively, it would seem logical to administer vitamin C preoperatively and postoperatively. Hanke[134] noted apparent acceleration of bone healing in fractures. following administration of vitamin C. Lauber, Bersin and Nafziger[135] report an increased demand for vitamin C in infection of a surgical nature. Myerson[136] suggests that vitamin C deficiency is one of the possible factors in hemorrhage following tonsillectomy.

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[132] Lanman, T. H., and Ingalls, T. H.: Vitamin C Deficiency and Wound Healing: Experimental and Clinical Study, Ann. Surg. **105:** 616 (April) 1937.

CONCLUSIONS

Vitamin C is definitely indicated as a therapeutic agent in the prevention and cure of scurvy. Whether vitamin C has a definite causal relationship to any other pathologic process is as yet uncertain. The vast amount of investigative and clinical research reported to date seems to be indicative of a further therapeutic role for vitamin C, though not enough progress has been attained to allow definite therapeutic conclusions to be formulated. Certain suggestive conditions may be briefly outlined when, aside from the prevention and cure of scurvy, vitamin C may be therapeutically indicated.

An ample supply is indicated in the diet of the pregnant woman and nursing mother. If the possibility of a prescorbutic state is accepted or if the existence of latent scurvy is recognized, it is imperative that adequate diagnostic methods and criteria be formulated in order to prevent the development of such states, which would be conditions of suboptimal nutrition and possibly deleterious to general health. We believe that fasting blood plasma values are to date the best method for determining the possibility of such a state. In order to prevent the occurrence of such a possibility, the minimal human requirement for vitamin C must be definitely known. Whether this requirement varies for different ages and different individuals is unknown.

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[133] Taffel, M., and Harvey, S. C.: Effect of Absolute and Partial Vitamin C Deficiency on Healing of Wounds, Proc. Soc. Exper. Biol. & Med. **38:** 418 (May) 1938.

[134] Hanke, H.: Experimentelle Untersuchungen über Beeinflussung der Knochenregeneration durch Vitamin C, Deutsche Ztschr. f. Chir. **245:** 530, 1935.

[135] Lauber, H. J.; Bersin, T., and Nafziger, H.: Der Ascorbinsäurebedark bei chirurgischen Infectionen, Klin. Wchnschr. **16:** 1274 (Sept.) 1937.

[136] Myerson, M. C.: Hemorrhage Following Tonsillectomy, Am. J. Surg. **36:** 151 (April) 1937.

The question of absorption following oral ingestion is of the utmost importance. Absorption has been entirely overlooked by most investigators and, when mentioned in the literature, has been discussed chiefly as a theoretical possibility. Little or nothing is known concerning the assimilation of vitamin C and its metabolism in abnormal and normal states. The implication that there is an increased need and an augmented metabolism for vitamin C in many infections is generally noted in all the numerous reports available. The exact proof for this important fact is lacking. How fever, toxemia and specific bacteria act on vitamin C in individual infections is still unknown. How vitamin C is absorbed, assimilated and utilized in specific infections is still unknown. The relation of vitamin C to the endocrines has been noted, and there are implications that it may be specifically related to hyperthyroidism, diabetes and Addison's disease. The exact facts are still lacking; this is more or less true of the other specific conditions here reviewed.

The failure of the oral administration of vitamin C in certain conditions in which its parenteral administration gives positive experimental or clinical results, and the positive results following its use in extremely large doses when its use in small doses was unavailing, are paradoxical questions which need elucidation.

Perhaps the value of a view on the therapeutic indications and implications for vitamin C at this time lies in its urge to spur us on to fill the gaps in our present knowledge which, it will be hoped, are as evident to the reader as they have been to the reviewers.

CHAPTER XXIII

THE CHEMISTRY OF VITAMIN D

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Studies of recent years have shown beyond a doubt that there are several chemically distinct forms of vitamin D. Nevertheless, this vitamin is still spoken of in the singular, chiefly as a convenience and because its multiple nature was not recognized for about a decade after the first form was discovered. Custom limits the application of the term vitamin D to antirachitic sterol derivatives and to the unidentified antirachitic components of fish oils and other foods which are supposed to belong to this group.

Of the several forms of vitamin D which have been recognized to date, two are known to be of prime importance in medicine. These are activated ergosterol and activated 7-dehydro-cholesterol. Other forms undoubtedly contribute to the total antirachitic effectiveness of certain agents, and it may be that the importance of some is greater than now appears. But for practical purposes, one may consider that all antirachitic medicines and foods owe their peculiar property chiefly to either or both of the two forms mentioned.

Activated ergosterol comes to the physician in several types of products which he may not always recognize as essentially identical. Ergosterol, activated by exposure to ultraviolet rays and dissolved in an oil, is viosterol in oil. Similar solutions are known abroad under several other trade names. Activated ergosterol separated from the unimportant by-products of irradiation and crystallized comprises one form of vitamin D in the pure state. This is known to English and American chemists as calciferol. The Germans sometimes call it vitamin D_2. Since ergosterol is the principal sterol of yeast, and probably the only activatable one present, it follows that irradiated yeast owes its antirachitic action to its content of calciferol. When irra-

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From the Research Laboratory, Mead Johnson & Co.

diated yeast is fed to cattle, the milk obtained is known as "metabolized" vitamin D milk. Since milk normally contains no important amount of vitamin D, the vitamin D of this special milk is essentially the calciferol which is transferred from the yeast. Thus the vitamin D of viosterol, irradiated yeast and yeast milk is one and the same; namely, calciferol.

Activated 7-dehydro-cholesterol has been identified, somewhat incompletely, as the form of vitamin D to which most other antirachitic agents owe their virtue. 7-Dehydro-cholesterol appears to be the principal activatable sterol, or provitamin, in cholesterol, the chief sterol of animal fats. Its active form thus comprises the vitamin D, which is produced in the skin, fur or feathers of animals exposed to sunlight or other sources of ultraviolet rays. For the same reason it is the form of vitamin D present in irradiated milk. It has been identified in certain fish oils and probably is the chief form, though certainly not the only form, of vitamin D that these oils contain. Since fish oils are almost universally used in poultry feeds, it appears that a substantial amount of this form of vitamin D finds its way into eggs, supplementing the supply produced by insolation of the feathers and skin of the birds. (Eggs are the only common food, except fish, naturally containing substantial amounts of vitamin D.) Fish oil concentrates are widely used to "vitaminize" milk, and the milk so treated is essentially the same as irradiated milk, but different from yeast milk, as regards the kind of vitamin D which it contains.

ACTIVATED ERGOSTEROL

ergosterol is the form of vitamin D best known to the chemist.[1] Its parent substance, or provitamin, ergosterol, is the characteristic sterol of fungi. Ergosterol was first obtained from mushrooms by Braconnot in 1811. It was rediscovered by several nineteenth century chemists and was particularly studied by Tanret between 1879 and 1908. Tanret named it ergostérine because of its occurrence in ergot and its similarity to the cholestérine of Chevreul. It is now prepared from yeasts and molds, in which fungi it occurs in amounts up to 2 per cent of the dry weight, the quantity varying with the species and conditions of culture. Its origin is obscure, but seemingly it is related more closely to the metabolism of carbohydrates than to that of fats.

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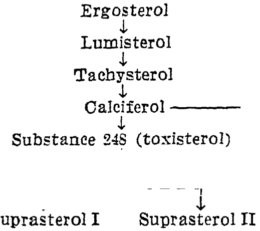
[1] In this paper, most of the references are to recent studies. References to the earlier chemical and physiologic investigations are given in the author's previous reviews: Bills, C. E.: Physiology of the Sterols, Including Vitamin D, Physiol. Rev. **15**: 1-97 (Jan.) 1935; The Multiple Nature of Vitamin D, Cold Spring Harbor Symp. Quant. Biol. **3**: 328-340, 1935 (symposium of July 27); New Forms and Sources of Vitamin D, J. A. M. A. **108**: 13-15 (Jan. 2) 1937. Fieser, L. F.: Chemistry of Natural Products Related to Phenanthrene, New York, Reinhold Publishing Company, 1936. Strain, W. H.: The Sterols, Bile Acids, and Related Compounds, chap. 15 of vol. II of Gilman's Organic Chemistry, New York, John Wiley and Sons, 1938. Sobotka, H.: Chemistry of the Sterids, Baltimore, Williams and Wilkins, 1938.

Ergosterol, $C_{28}H_{43}OH$, forms small colorless crystals usually containing some water of crystallization. The melting point varies with the water content, being 166° C. for the nearly anhydrous sterol. Polariscopic measurements show $[\alpha]^{20}_{5461} = -171°$ and $[\alpha]^{20}_{D} = -132°$ in chloroform. Three double bonds are present. The heat of combustion is 9,950 calories_{15} per gram. Commercial ergosterol contains about 5 per cent of α-dihydro-ergosterol, an inert sterol which always accompanies ergosterol in fungi and which is unaltered by irradiation. Ergosterol is insoluble in water, sparingly soluble in oils and freely soluble in most of the common organic solvents.

Although ergosterol is colorless, i. e., nonabsorbing of visible light, it absorbs certain ultraviolet wavelengths as powerfully as intense dyes do in the visible parts of the spectrum. It exhibits four narrow absorption bands, with maximums at wavelengths 260, 270, 282 and 293.5 millimicrons respectively. The phenomena of activation are closely associated with these bands of absorption.

When ergosterol is exposed to ultraviolet radiations of the wavelengths which it absorbs, a transformation into vitamin D (calciferol) begins at once. However, the ergosterol, even under the most favorable conditions, never gives a 100 per cent yield of calciferol. The transformation proceeds in overlapping steps, with the formation of a series of products of which calciferol is not the last. This intricate system has been studied most extensively by Windaus and his associates at

Goettingen and can be illustrated conveniently, if not too well, by the following modification of a scheme given by Setz.



At no stage during the irradiation is any one of these substances present alone. More radiant energy seems to be required to decompose calciferol than to produce it, so that when the irradiation is not unduly prolonged calciferol is the chief product. Under the best conditions it may comprise nearly 50 per cent of the total, with tachysterol and lumisterol accounting for most of the remainder. Calciferol, lumisterol and the two suprasterols have been isolated in crystalline form. Tachysterol has been separated as a crystalline 3,5-dinitro-4-methyl benzoate. Substance 248, sometimes called toxisterol, has not been obtained pure.

The reactions are purely photochemical ones, little influenced by temperature. They can take place in boiling solvents or at room temperature or at the temperature of liquid air. Nothing is added to the ergosterol molecule and nothing leaves it. There is no polymerization. The changes are merely rearrangements within the molecule. Any wavelength of light that is absorbed by ergosterol can initiate the transformations. Activation takes place more effectively in solutions than in solid masses. Solutions that are agitated during irradiation are activated better than those which remain quiet. The presence of more than traces of air is detrimental. There is a specific solvent effect, in accordance with which various solvents which are satisfactory as to transparency and nonreactiveness are nevertheless unequally effective in promoting the activation of ergosterol dissolved in them.

To comprehend the molecular changes that occur when ergosterol and other D provitamins are irradiated, one must examine the structural formulas of these sterols. The accompanying figures and discussion are necessarily limited, though the field of sterol chemistry is wide.[1]

Figure 1 shows the accepted sterol ring structure, with a side chain as in ergosterol. The ring system is a phenanthrene-cyclopentane complex, the same as in many of the sex hormones. It is, incidentally, one of the biggest aromatic nuclei with which the animal body has to deal. Hydrocarbons of this type are formed when sterols are subjected to dehydrogenation with suitable reagents, such as selenium.

The prototype of all sterols is cholesterol, shown in figure 2. Ergosterol (fig. 3) differs from cholesterol in its side chain, which contains a double bond at C_{22} and a methyl group at C_{24}, and in its nucleus, which contains two double bonds instead of one. In hydroaromatic structures as complicated as the sterols, the possibilities of molecular rearrangement are great, yet most of those which are known to occur are *cis-trans* changes at C_3, C_5, C_9 or C_{10}. The spatial geometry of these changes is not perfectly understood, and structural formulas as ordinarily written do not show them.[2]

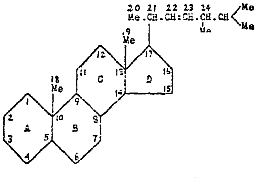


Fig. 1.—The sterol ring structure. Side chain shown as in ergosterol.

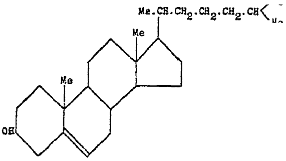


Fig. 2.—Cholesterol.

In all naturally occurring sterols (but not in the sterol derivatives called vitamin D), the hydroxyl group at C_3 is *cis* to the methyl group at C_{10}. It is a rule that this configuration exists when a sterol is capable of forming a precipitate with digitonin—the familiar analytical reaction by means of which cholesterol and ergosterol are determined. Since none of the irradiation products of ergosterol reacts with digitonin, it is assumed that in them the OH at C_3 is *trans* to the CH_3 at C_{10}. This rearrangement is probably the first change brought about by the activating rays and perhaps the only difference between ergosterol and lumisterol. Except for this difference, and the possibility of another steric difference at C_9, ergosterol and lumisterol are identical. Figure 3 is the formula for each of them, and also for pyro-calciferol and iso-pyro-calciferol, two sterols which result from heating calciferol. All four differ from one another only as *cis-trans* isomers, indistinguishable in ordinary, two-dimensional formulas.[2]

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[2] Schoenheimer, Rudolf, and Evans, E. A., Jr.: Allocholesterol and Epiallocholesterol, J. Biol. Chem. **114**: 567-582 (July) 1936. Windaus, Adolf, and Dimroth, K.: Die Konstitution des Lumisterins und der Erhitzungsprodukte des Vitamins D_2 (Calciferols), Ber. deutsche chem. Gesellsch. **70**: 376-379 (Feb.) 1937.

The change from lumisterol to tachysterol involves a deeper alteration of the molecule. The structural formula of tachysterol is not known with certainty, but since tachysterol and calciferol yield the same dihydro derivative[3] the two must have the same carbon skeleton. Tachysterol, like calciferol which follows it, has four double bonds instead of the three exhibited by lumisterol and ergosterol. The fourth double bond arises by a rupture of ring B. Tachysterol was given its name in recognition of the speed with which it enters certain reactions. A tentative structural formula, suggested by Lettré, is given in figure 4.

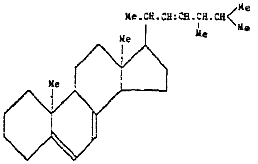

Fig. 3.—Ergosterol, lumisterol, pyro-calciferol and iso-pyro-calciferol.

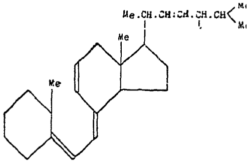

Fig. 4.—Tachysterol (a tentative formula).

In the change from tachysterol to calciferol, the molecule returns to a relatively stable form. The formula may be written either as in figure 5, which emphasizes the relation of calciferol to the sterols (steroid formula), or as in figure 6, which emphasizes the broken ring B (stilbenoid formula). Different as the two formulas may look at first glance, they are actually the same. Figure 5 is the conventional representation, but figure 6 shows more clearly that the vitamin is not a sterol in the strictest sense, albeit a sterol derivative.

Irradiation of calciferol (i. e., overirradiation of ergosterol) occasions the formation of substance 248 and the two suprasterols. Substance 248 derives its name from its single intense absorption band, the maximum of which is at wavelength 248 millimicrons and is

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3. Müller, M.: Zur Kenntnis des Vitamin D und seiner thermischen und photochemischen Umwandlungsprodukte, Ztschr. f. physiol. Chem. **233**: 223-234 (June 7) 1935.

thus outstandingly different from the longer wavelength maximums of the irradiation products preceding it. Sometimes it is called toxisterol, in recognition of the toxic-calcifying properties which it exhibits out of all proportion to its antirachitic activity, if any. Toxisterol is formed most readily when alcohol is the solvent in which the overirradiation is conducted. When ether is the solvent it may not appear in detectable amounts. If it is formed at all it must immediately pass over to the suprasterols, into which it also passes when irradiation of alcoholic solutions is sufficiently prolonged. The substance has never been isolated, so practicaly nothing is known about it chemically.

The toxic properties peculiar to one particular brand of irradiated ergosterol solution were the subject of numerous reports in the medical literature of 1928 to 1930. The German manufacturer of the offending product had obtained two patents, one British[4] and the other American,[5] the specifications of which are most revealing. They call for the irradiation of ergosterol to be continued just until the absorption spectrum of what is now recognized as toxisterol attains its maximum! The mistake was clearly the result of assuming, in the absence of proper bio-assays, that the spectrographically most conspicuous irradiation product was the vitamin. That this is not so was known, even in 1928, from a study[6] conducted in this laboratory in which substance 248 was recognized as a decomposition product of the vitamin.

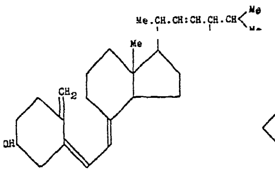

Fig. 5.—Calciferol (vitamin D_2). Usual, or steroid, formula.

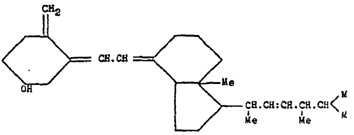

Fig. 6.—Calciferol, stilbenoid formula.

Whatever may be the number of double bonds in toxisterol, it appears that in suprasterol I the number is reduced from four to three. This results from a closure of the break in ring B, but the closure which in this case is brought about by prolonged exposure to ultraviolet rays is different from that which is produced when the two pyro-calciferols are made from calciferol by the action of heat. In suprasterol I the restored ring B may be five sided rather than six sided (fig. 7). Less is known of suprasterol II; the distinction I or II is arbitrary and does not indicate sequence of formation. Even the number of double bonds, to say nothing of their position, is unknown.[3]

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[4] British Patent 296,093, Nov. 15, 1928: Improvements in the Photochemical Production of Vitamin D from Ergosterol.

[5] U. S. Patent 1,896,191, Feb. 7, 1933: Production of Vitamin D.

[6] Bills, C. E.; Honeywell, E. M., and Cox, W. M., Jr.: Antiricketic Substances: IX. Quantitative Biophysical Studies on the Activation of Ergosterol, J. Biol. Chem. **80:** 557-563 (Dec.) 1928.

In the accompanying table is a summary of properties of the irradiation products of ergosterol, including also the properties of the pyro-calciferols.

ACTIVATED 7-DEHYDRO-CHOLESTEROL

For several years after the activatability of ergosterol was known there prevailed a hypothesis, disproved by Waddell[7] in 1934, that traces of ergosterol comprised the provitamin to which the common sterols of animals and plants owed their activatability. This concept was based on the fact that the common sterols usually show the characteristic absorption spectrum of ergosterol more or less in proportion to their activatability. The possibility had been overlooked that there might exist other D provitamins having the same absorption spectrum as ergosterol.

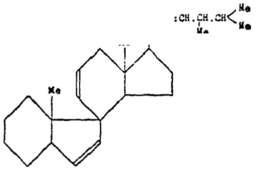

Fig. 7.—Suprasterol I (a tentative formula).

On theoretical grounds it appeared that 7-dehydrocholesterol might be such a provitamin. This compound was prepared in the following semisynthesis by Windaus, Lettré and Schenck[8] and was found to be highly activatable. Cholesteryl acetate was oxidized by chromic acid to 7-keto-cholesteryl acetate. This was reduced by

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[7] Waddell, J.: The Provitamin D of Cholesterol: I. The Antirachitic Efficacy of Irradiated Cholesterol, J. Biol. Chem. **105**: 711-739 (July) 1934.

[8] Windaus, Adolf; Lettré, H., and Schenck, F.: Ueber das 7-Dehydro-Cholesterin, Ann. **520**: 98-106 (Sept.) 1935.

Physicochemical Properties of the Irradiation Products of Ergosterol and of the Thermal Decomposition Products of Calciferol

					Specific Rotation, $[\alpha]_D$		
Substance	Melting Point, Degrees C.	Digitonin Reaction	Position* of Absorption Maximums, Millimicrons	Double Bonds	Rotation, Degrees	Solvent	Tempera ture, Degrees C.
Ergosterol	166	Positive	260, 270, **282**, 293.5	3	−132	Chloroform	20
Lumisterol	118	Negative	265, **280**	3	+192	Acetone	19
Tachysterol	?	Negative	268, **280**, 294	4	− 70	Benzine	18
Calciferol	116	Negative	265	4	+106	Alcohol	20
Toxisterol	?	Negative	248	?	?		
Suprasterol I	104	Negative	Only in far ultraviolet	3	− 76	Chloroform	18
Suprasterol II	110	Negative	Only in far ultraviolet	?	+ 63	Chloroform	19
Pyro-calciferol	95	Negative	274, **284**, 296	3	+502	Alcohol	20
Iso-pyro-calciferol	115	Partial	265, **280**, 294	3	+332	Chloroform	20

* The position of the most important band is indicated by the use of bold face type.

aluminum isopropylate to 7-hydroxy-cholesterol, the di-benzoate of which, when strongly heated, gave the mono-benzoate of 7-dehydro-cholesterol. From this the free sterol (fig. 8) was obtained by saponification.

In at least one instance, 7-dehydro-cholesterol has been chemically identified as the provitamin D of ordinary cholesterol. Windaus and Bock[9] isolated it from the sterol of hog skin. Boer, Reerink, van Wijk and van Niekerk[10] also isolated it from a crude cholesterol of unstated origin. However, ergosterol itself sometimes occurs in animal cholesterol, as was shown by Windaus and Stange[11] in eggs. This is probably an exceptional instance, associated with the fact that hens, unlike the mammals that have been investigated, absorb ergosterol from their food. The presence of ergosterol in eggs therefore seems casual, especially since the vitamin D of eggs is ordinarily not the ergosterol vitamin D, a form which, for birds, is relatively ineffective in comparison with the vitamin D from 7-dehydro-cholesterol.[12] The chief evidence that 7-dehydro-cholesterol is the usual provitamin D of cholesterol is found in experiments with rats and chickens which show, in a word, that the vitamin D produced by irradiating the cholesterol from butter, brains, fish oils and other sources has the biologic properties of activated 7-dehydro-cholesterol rather than those of activated ergosterol.

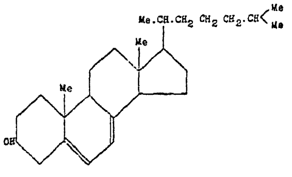

Fig. 8.—7-Dehydro-cholesterol.

Activated 7-dehydro-cholesterol, prepared by irradiating the synthetic provitamin, has recently been obtained in crystalline form by Schenck.[13] The crystalline vitamin has not yet been obtained from natural sources, but crystalline esters of it have been obtained from tuna [14] liver (species not specified) and halibut [15] liver oils. These esters resemble the corresponding esters of the artificially made vitamin, but the fish oil vitamin liberated from the esters could not be crystallized.

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[9] Windaus, Adolf, and Bock, F.: Ueber das Provitamin aus dem Sterin der Schweineschwarte, Ztschr. f. physiol. Chem. **245:** 168-170 (Jan. 29) 1937.

[10] Boer, A. G.; Reerink, E. H.; van Wijk, A., and van Niekerk, J.: A Naturally Occurring Chicken Provitamin D, Proc. Acad. Sc. Amsterdam **39:** 622-632 (May) 1936.

[11] Windaus, Adolf, and Stange, O.: Ueber das Provitamin des Eiersterins, Ztschr. f. physiol. Chem. **244:** 218-220 (Nov. 26) 1936.

[12] Bills, C. E.; Massengale, O. N.; Imboden, Miriam, and Hall, Helen: The Multiple Nature of the Vitamin D of Fish Oils, J. Nutrition **13:** 435-452 (April 10) 1937.

This fact, and also the fact that the potency appears to be significantly less than that of the crystalline vitamin, suggest that the vitamin preparations from fish oils were not pure, even though the evidence is good that they consisted chiefly of activated 7-dehydrocholesterol.

The formula of activated 7-dehydro-cholesterol is shown in figure 9, from which its close relationship to calciferol (fig. 5) is evident. It is, in fact, a demethyl-dihydro-calciferol, just as its provitamin (fig. 8) is a demethyl-dihydro-ergosterol. Its absorption spectrum resembles that of calciferol, just as the absorption spectrum of its provitamin resembles that of ergosterol. Its antirachitic effectiveness is the same as that of calciferol for rats but many times greater for chickens.

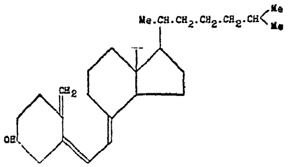

Fig 9.—Activated 7-dehydro-cholesterol (vitamin D_3).

In the German literature, activated 7-dehydro-cholesterol is called vitamin D_3. Calciferol is vitamin D_2. But there is no vitamin D_1, this term having been preempted for a lumisterol-calciferol mixture originally mistaken for the pure vitamin. Vitamin D_4 is the name applied to activated 22-dihydro-ergosterol, to be described.

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[13] Schenck, F.: Ueber das kristallisierte Vitamin D_3, Naturwissenschaften **25**: 159 (March 5) 1937.

[14] Brockmann, Hans: Die Isolierung des antirachitischen Vitamins aus Thunfischleberöl, Ztschr. f. physiol. Chem. **241**: 104-115 (July 2) 1936. Haslewood, G. A. D., and Drummond, J. C.: Antirachitic Substance from Tunny Liver Oil, Chem. and Ind. **55**: 598-599 (July 24) 1936. Simons, E. J. H., and Zucker, T. F.: An Antirachitic Substance from Tuna Liver Oil, J. Am. Chem. Soc. **58**: 2655 (Dec.) 1936.

[15] Brockmann, Hans: Die Isolierung des antirachitischen Vitamins aus Heilbutt-leberöl, Ztschr. f. physiol. Chem. **245**: 96-102 (Jan. 29) 1937.

LESS IMPORTANT FORMS OF VITAMIN D

A provitamin D which is intermediate in structure between ergosterol and 7-dehydro-cholesterol was prepared by Windaus and Langer[16] by saturating the double bond in the side chain of ergosterol. Ergosteryl acetate was heated with maleic anhydride to form an addition product in which the two double bonds in ring B were saturated. The double bond at C_{22} was then hydrogenated, and the maleic anhydride was thermally split from the molecule, thus restoring the double unsaturation in ring B which is essential for activation. The free provitamin, known as 22-dihydro-ergosterol (fig. 10), was then obtained by saponification of the ester.

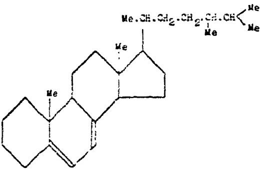

Fig. 10.—22-Dihydro-ergosterol.

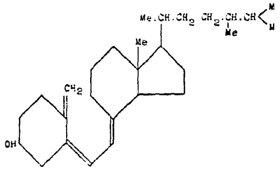

Fig. 11.—Activated 22-dihydro-ergosterol (22-dihydro-calciferol, or vitamin D_4).

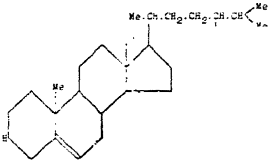

Fig. 12.—Sitosterol.

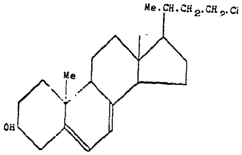

Fig. 13.—7-Dehydro-sitosterol.

By exposure to ultraviolet rays, this is activated to the corresponding vitamin, which undoubtedly is 22-dihydro-calciferol (fig. 11). This product has been obtained in crystalline form by Windaus and Trautmann.[17] It has the same absorption spectrum as calciferol and demethyl-dihydro-calciferol. Its provitamin has the same absorption spectrum as that of the other two provitamins. It appears to be slightly less antirachitic for rats than the other two D vitamins, and for chickens its activity, per rat unit, is intermediate.[18]

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16. Windaus, Adolf, and Langer, R.: Ueber das 22-Dihydro-Ergosterin, Ann. **508:** 105-114 (Dec.) 1933.

17. Windaus, Adolf, and Trautmann, G.: Ueber das krystallisierte Vitamin D_4. Ztschr. f. physiol. Chem. **247:** 185-188 (June 15) 1937.

18. McDonald, F. G.: The Multiple Nature of Vitamin D: III. Irradiated 22-Dihydro-Ergosterol, J. Biol. Chem. **114:** lxv (May) 1936.

22-Dihydro-calciferol, or vitamin D_4, has not been isolated from natural sources. Possibly its provitamin occurs in the phytosterols of the plant kingdom. If so, this form of vitamin D is produced when vegetable products are irradiated, and it may come to the interest of the physician in such forms as irradiated rolled oats and breakfast foods.

By reactions analogous to those used in converting cholesterol into 7-dehydro-cholesterol, Wunderlich[19] has converted sitosterol (fig. 12) into 7-dehydro-sitosterol (fig. 13). This has the "ergosterol" absorption bands and can be activated to a vitamin, supposedly of the formula shown in figure 14. This vitamin, differing by a side chain ethyl group from activated 7-dehydro-cholesterol, appears to be much less antirachitic than the latter for rats. However, the two have not been compared in the pure state. Like vitamin D_4, this form of vitamin D may be produced when vegetable products are irradiated.

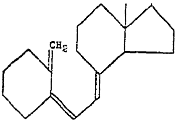

Fig. 14.—Activated 7-dehydro-sitosterol.

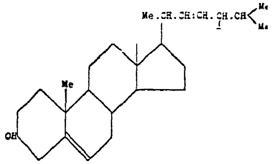

Fig. 15.—Stigmasterol.

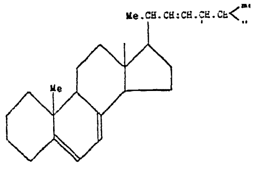

Fig. 16.—7-Dehydro-stigmasterol.

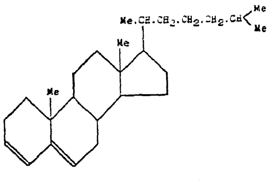

Fig. 17.—Cholesterilene.

Again, by the same reactions, Linsert[20] has converted stigmasterol (fig. 15) into 7-dehydro-stigmasterol (fig. 16), which exhibits an absorption spectrum "very much like" that of ergosterol. Chemically, it differs from ergosterol only in that it has an ethyl group at C_{24}

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[19] Wunderlich, W.: Ueber das 7-Dehydro-sitosterin, Ztschr. f. physiol. Chem. **241**: 116-124 (July 2) 1936.

[20] Linsert, O.: Ueber das 7-Dehydro-Stigmasterin, Ztschr. f. physiol. Chem. **241**: 125-128 (July 2) 1936.

instead of a methyl group. When irradiated, it most surprisingly does not become antirachitic, or at most very slightly so. This anomalous behavior, if true, is an instance of the fine specificity with which small molecular differences influence the physiologic properties of substances. It would be a matter of considerable theoretical interest to ascertain whether the reported failure of the expected new vitamin D is evident when the irradiated substance is given intravenously as well as when given by mouth.

Years ago, I observed that treatment of cholesterol with fullers' earth resulted in a slight activation. Yoder[21] has traced this reaction to the formation of cholesterilene, the probable formula of which (fig. 17) has recently been given by Stavely and Bergmann.[22] Cholesterilene sulfonic acid and its salts are definitely, though not powerfully, antirachitic for rats, and it has been reported that their effectiveness for chicks, per rat unit, is somewhat greater than that of cod liver oil. It will be observed that cholesterilene differs greatly in structure from the other forms of vitamin D.

Up to this point I have considered five forms of vitamin D, the chemical structures of which are well understood. Four of these are the active irradiation products of ergosterol, 7-dehydro-cholesterol, 22-dihydro-ergosterol and 7-dehydro-sitosterol respectively. The fifth, cholesterilene sulfonic acid, requires no irradiation. There are still other forms, as yet not understood chemically, but which deserve mention in passing.

Sixth: McDonald has found that by the irradiation of 7-hydroxy-cholesterol (or an impurity associated therewith) a slight antirachitic potency is developed. This may or may not be the same kind of vitamin D as that which is developed when cholesterol, freed from its normal provitamin and subsequently heated to produce a new provitamin, is irradiated.[1]

Seventh: Cholesterol freed from its normal provitamin but not heated to produce the new one gives on irradiation a vitamin D which is less effective for chickens, per rat unit than the vitamin D which is formed by irradiating either unpurified cholesterol or heat-treated purified cholesterol.[23]

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[21] Yoder, L.: The Chemical Activation of Sterols: I. The Nature of the Floridin Activation of Cholesterol, J. Biol. Chem. **116**: 71-80 (Nov.) 1936.

[22] Stavely, H. E., and Bergmann, W.: The Chemistry of Unsaturated Steroids: I. The Constitution of Cholesterilene, J. Org. Chem. **1**: 567-574 (Jan.) 1937.

Eighth: McDonald and I found that ergosterol, when suitably treated with nitrites, gives an active product of low potency. It is believed to be different from the ordinary forms of vitamin D which are destroyed by nitrites.[1]

Ninth: Weinhouse and Kharasch [24] have produced a form of vitamin D by irradiating the heated reaction product of 7-keto-cholesteryl acetate and isobutyl-magnesium bromide.

Tenth: Windaus and Trautmann,[17] in a footnote, mention "other provitamins, e. g., 22,23-oxido-ergosterol, which, however, are only feebly active after irradiation."

To correct a somewhat prevalent wrong impression, it may be pointed out that the kind of vitamin D which is produced by irradiating a given provitamin is not determined or even influenced by the conditions of irradiation. For example, from ergosterol, calciferol is the only kind of vitamin D produced. Whether long wavelength, short wavelength or mixed wavelength radiation is employed, as in different commercial processes, the end product is the same as regards the vitamin. The only differences are in the relative amounts of by-products, chiefly in the lumisterol and tachysterol, which are unimportant physiologically in the amounts present. Avoidance of the undesirable toxisterol is a matter of choice of solvent and duration of exposure.[1] Processes in which electron bombardment is substituted for ultraviolet irradiation produce the same kind of vitamin D, although they do so inefficiently because of extensive decomposition.[25]

Little has been said here with regard to the D vitamins of fish oils, which comprise by far the largest source of antirachitic medication for both man and animals. Demethyl-dihydro-calciferol (vitamin D_3), as already stated, appears to be the principal form of vitamin D present. However, the work of Bills, Massengale, Imboden and Hall [12] shows that more than one

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23. Hathaway, M. L., and Lobb, D. E.: The Provitamin D of Heat-Treated Cholesterol, J. Biol. Chem. **113:** 105-110 (Feb.) 1936.

24. Weinhouse, S., and Kharasch, M. S.: Provitamin D Activity and Structure—Addition of Grignard Reagents to 7-Ketocholesteryl Acetate. J. Org. Chem. **1:** 490-495 (Nov.) 1936.

25. Hoffman R. M., and Daniels, Farrington: The Formation of Vitamin D by Cathode Rays, J. Biol. Chem. **115:** 119-130 (Aug.) 1936.

kind of vitamin D occurs in the liver oils of various species of fish. Progress in separating and identifying the natural vitamins D may be expected from studies now under way in which the molecular distillation technic of Hickman [26] is applied to liver oils. Evidence has already been obtained that cod liver oil contains several kinds of vitamin D, which differ in boiling point and hence, supposedly, in the length of their side chain. The lowest-boiling form has been further differentiated by the dual assay method with rats and chickens.[27]

SUMMARY

The properties of vitamin D are exhibited by at least ten different sterol derivatives. Five of these are well understood chemically and five are distinguished by fragmentary chemical and physiologic differences. The two that are of prime importance are the activation products of ergosterol and 7-dehydro-cholesterol respectively. A few of the others may have some practical significance, though most of them are merely of theoretical interest, illustrating the concept of the multiple nature of vitamin D.

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[26] Hickman, K. C. D.: Molecular Distillation: Apparatus and Methods, Indust. & Engin. Chem. **29**: 968-975 (Sept.) 1937.

[27] Bills, C. E.; Massengale, O. N.; Hickman, K. C. D., and Gray, E. LeB.: A New Vitamin D in Cod Liver Oil, J. Biol. Chem. **126**: 241-244 (Nov.) 1938.

CHAPTER XXIV

PHYSIOLOGY AND PATHOLOGY OF VITAMIN D

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BOSTON

Vitamin D prevents or cures rickets. Until recently this factor was thought to be a single substance. The active compound formed by the irradiation of ergosterol was considered identical with the antirachitic agents present in natural products or formed in animals or foods by ultraviolet irradiation. It was found, however, that different sources of vitamin D might have different relative values, depending on whether the products were tested with rats or with chickens. It was found also that several different sterols, carefully purified, could be activated by various methods. The multiple nature of vitamin D thus became established, and Bills recently has described ten different substances which are capable of exerting a vitamin D effect. As more information becomes available regarding the chemical nature of the different forms of vitamin D, there may be afforded a clearer understanding of the physiologic and pharmacologic aspects of each active substance. At the present time, however, vitamin D can be detected only by its effect in the prevention or alleviation of rickets. Thus it happens that different sources of vitamin D have qualitatively the same effect on the animal body.

RICKETS

Rickets is a condition made manifest by defects in the calcification of growing bones. It may be recognized by roentgenologic examination of the bones clinically or in experimental animals. In the latter, interpretation of the roentgenograms may be uncertain. Final resort, as to diagnosis, is the histologic findings. When the diagnosis is established, the degree of rickets may be quantitated by chemical analysis of the bone ash.

From the Department of Pediatrics of the Harvard University Medical School and the Infants' and Children's Hospitals.

The sequence of events in the production of normal and of rachitic bone has been epitomized by Wolbach.[1] Growth in the length of the long bones occurs by endochondral formation of bone. In the normal tibia of the growing rat, for example, the epiphysial cartilage at the upper end of the shaft is a narrow plate. This is firmly supported by bone, in the form of transverse trabeculae or a thin fenestrated plate, on the epiphysial side, and it is uniformly penetrated by capillaries on the diaphysial side. Growth occurs by the continuous proliferation of cartilage cells, which are arranged in orderly columns on the epiphysial side, and by the simultaneous degeneration of the matured cartilage cells on the diaphysial side. In the spaces left by the degeneration and disappearance of the cartilage cells there appear capillaries and bone-forming cells, or osteoblasts. These are responsible for the deposition of the bony matrix. The growth of bone by endochondral formation of bone, according to Wolbach, is achieved "by a continuously retreating gap in the continuity of tissues, maintained on the epiphysial side by continuous renewal of cartilage cells and on the diaphysial side repaired by vascular outgrowth comparable to repair of any defect of tissues by the process of organization or granulation tissue formation. In normal growth there presents on the diaphysial side of the narrow cartilage a continuous layer of clear or empty cartilage cells forming an almost straight line."

The first histologic evidence of rickets is the cessation of the degenerative sequences of the cartilage cells. The layer of clear cells does not form, and consequently there is no ingrowth of capillaries and osteoblasts. If the degree of rickets is slight, there is a moderate increase in the width of the epiphysial cartilage. Because the cessation of degenerative sequence in the cartilage cells does not occur simultaneously in all the affected cells, the epiphysial cartilage presents an irregular border on the diaphysial side. The width of the cartilage increases because of continued activity of the proliferative zone and the survival of cells on the diaphysial side.

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[1] Shohl, A. T., and Wolbach, S. B.: Rickets in Rats: XV. The Effect of Low Calcium-High Phosphorus Diets at Various Levels and Ratios upon the Production of Rickets and Tetany, J. Nutrition **11**: 275 (March) 1936.

After the cessation of degenerative changes in the cartilage cells of the rachitic bone, calcification of the cartilaginous matrix ceases, and osteoid material accumulates around the capillaries of the diaphysis. In advanced rickets some changes occur that are probably brought about by the pressure of weight bearing. The uncalcified cartilage of the diaphysial border often is stratified transversely in places, and the osteoid material, which is also uncalcified and which increases in amount with the duration of the dietary deficiency, becomes molded. In long-continued rickets there may be a disappearance of the cancellous bone of the diaphysis and a marked resorption of cortical bone. "The degree or severity of rickets may be recorded on the basis of the prominence of anatomical changes. Obviously two factors enter into the production of the pathological picture; the duration of the deficient diet and the degree of the deficiency." [1]

The changes in the bones in rickets result in those gross manifestations which are recognized clinically; [2] namely, enlargement of the wrists, knees and ankles, bowed legs, beading of the ribs and the rachitic rosary, Harrison's groove and craniotabes.

When vitamin D is given to rachitic animals, calcification begins again at the line of provisional calcification. The first histologic evidence of repair is the presence along the diaphysial border of degenerated cartilage cells. This effect is visible at the end of twenty-four hours and is accompanied by extensive vascular penetration within forty-eight hours. The penetration of blood vessels permits the deposition of the bone-forming salts. There is thus produced the so-called line test for healing. The mass of irregular cartilage cells becomes arranged into short, orderly, parallel columns of a few cells. Osteoid material is no longer formed. A remarkable degree of repair takes place, but for a long time the expert pathologist can detect traces of the former abnormality.

It has now become generally recognized that there is no fundamental pathologic condition in the rachitic bone which prevents its calcification. Shipley [3] reported in 1924 that slices of rachitic bone and cartilage became calcified when placed in normal blood serum. Later Shipley, Kramer and Howland[4] showed that suitable solutions of inorganic salts would bring about a similar result. This has been confirmed by Robison and Soames.[5] Further, as shown by Harris,[6] there is no reason to believe that vitamin D acts directly on bone cells to promote calcification. The action of vitamin D, instead, is so to alter the body fluids that bone salts may be incorporated into the growing bone. Rickets may be regarded as a fault in the metabolism of calcium and phosphorus. In this sense, rickets is the cause and not the result of the bony lesion.

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[2] Hess, Alfred: Rickets, Osteomalacia and Tetany, Philadelphia, Lea & Febiger, 1929. György, Paul: Umsatz der Erdalkalien (Ca, Mg) und des Phosphats. Handb. d. norm. u. path. Physiol. **16:** 1555-1641, 1931; Rachitis und Tetanie, Berlin, Julius Springer, 1929.

[3] Shipley, P. G.: Healing of Rickety Bones in Vitro, Bull. Johns Hopkins Hosp. **35:** 304 (Sept.) 1924.

CHEMISTRY OF CALCIUM AND PHOSPHORUS PRECIPITATION

Whatever may be the cause of the defect in metabolism which leads to rickets, the pathogenesis lies in the condition of the Ca^{++} and $(PO_4)^{---}$ in the blood serum.[7] Here is found the material available for excretion by the intestine or kidney or for deposition in bone or other tissues of the body. It has been mentioned that minerals will precipitate in rachitic cartilage in vitro either in normal serum or in properly constituted inorganic solutions. This does not imply that the formation of bone is an inorganic process. It takes place in the living cell, and the proper type of cartilage degeneration is essential to the entrance of the blood vessels. What takes place in the space between the cells is quite obscure, but certainly the proper inorganic environment is essential to deposition. It can best be expressed in physiochemical terms.[8] Only when the adequate concentration of calcium, phosphate and carbonate ions is present can precipitation of the salt take place. This complex salt is a dahllite, $n\text{Ca}_3(\text{PO}_4)_2\text{CaX}$ in which the n varies between 2 and 3 and the X may be CO_3, OH or F. It may be regarded as a solid solution of

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[4] Shipley, P. G.; Kramer, B., and Howland, J.: Calcification in Vitro, Biochem. J. **20**: 379 (no. 2) 1926.

[5] Robison, Robert, and Soames, Katharine M.: Possible Significance of Hexosephosphoric Esters in Ossification: Calcification in Vitro, Biochem. J. **24**: 1922 (no. 6) 1930.

[6] Harris, L. J.: Mode of Action of Vitamin D: Parathyroid Theory; Clinical Hypervitaminosis, Lancet **1**: 1031 (May 14) 1932.

[7] Schmidt, C. L. A., and Greenberg, D. M.: Occurrence, Transport and Regulation of Calcium, Magnesium and Phosphorus in Animal Organism, Physiol. Rev. **15**: 297 (July) 1935.

[8] Hastings, A. B.: Factors Governing the Calcium Equilibria in the Body, New England J. Med. **216**: 377 (March 4) 1937.

$CaCO_3$ in $Ca_3(PO_4)_2$. The ratio of two mols of $Ca_3(PO_4)_2$ to one mol of $CaCO_3$ is practically constant for adult bone, which by analysis gives 12 per cent $CaCO_3$ and 88 per cent $Ca_3(PO_4)_2$.

By the use of a frog heart method for the determination of ionized calcium, McLean and Hastings[9] were able to show that the larger part of the serum calcium was combined with protein. Of the usual 10 mg. per hundred cubic centimeters of serum, about 4.5 mg. or 1.1 millimols are ionized. According to their calculations not over 5 per cent of the total calcium can be in diffusible nonionized form.

The composition of the salt precipitated from a solution containing Ca^{++}, $(PO_4)^{---}$ and $(CO_3)^{--}$ gradually increases in CO_2 content. Logan and Taylor[10] have shown that the solubility product varies with the amount of substrate present and that a higher concentration is necessary to initiate the formation of the precipitate than to permit its subsequent formation. Thus, although the concentrations of these ions at the site of calcification are as yet unknown, the advances that have recently been made in the quantitation of Ca^{++}, $(PO_4)^{---}$ and $(CO_3)^{--}$ and the solubility product constant necessary for precipitation in bone bring chemical explanation much closer.

PHOSPHATASE

The enzyme phosphatase discovered by Robison[11] has the property of splitting organic phosphorus compounds such as hexose phosphate into inorganic phosphate. This enzyme occurs in largest amount in the cartilage but appears also in the kidney, bowel and blood serum. Just what the function of phosphatase in the serum may be is not known, for the acid soluble phosphorus is present almost entirely as inorganic phosphate. Kay[12] regards it as a "leakage" from tissues of higher content. It may deal with the excretion of phosphates by way of the kidney or bowel or the metabolism within the cell. Such a liberation of phosphate may be a step in the mechanism of carbohydrate metabolism or in muscle function. Certainly, if phosphorus esters were present in bone tissue, the local increase of inorganic phosphate would be an important step in the precipitation of calcium phosphate. However the phosphatase acts, there is no question that in diseases of the bone, especially resorptive ones in which osteoblastic activity is increased, the phosphatase of the serum is increased. Such is the case in rickets. Its increase is perhaps the first definite evidence of development of the rachitic condition, antedating roentgenogram changes and alterations in the blood serum phosphate. The content is high in active rickets. When vitamin D is administered it decreases toward normal, but more slowly than the other values; the phosphatase may not reach a normal content for several months after there is evidence of healing.[13] The increase of phosphatase in low phosphorus rickets thus acts as a protective mechanism.

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[9] McLean, F. C., and Hastings, A. B.: The State of Calcium in the Fluids of the Body: I. The Conditions Affecting the Ionization of Calcium, J. Biol. Chem. **108**: 285 (Jan.) 1935.

[10] Logan and Taylor: Cited by Hastings.[8]

[11] Robison, Robert: The Significance of the Phosphoric Esters in Metabolism, Herter Lectures, New York, New York University Press, 1932.

[12] Kay, H. D.: Plasma Phosphatase: Enzyme in Disease, Particularly in Bone Disease, J. Biol. Chem. **89**: 249 (Nov.) 1930.

HYPERVITAMINOSIS D

When huge excesses of vitamin D are given, there results a definite pathologic condition called hypervitaminosis D.[14] It is an exaggerated form of the physiologic or therapeutic effect of this vitamin. The calcium and the phosphate level in the blood are raised above normal. Calcification takes place at an increased rate, so that on roentgenologic or histologic examination the deposition of minerals at the provisional zone of calcification is usually dense. When the dose is sufficiently large, about a thousand times the therapeutic dose, the deposition of minerals at the epiphyses is made at the expense of that in the shaft. The balances of calcium and phosphorus become negative.[15] Metastatic calcification now takes place. The organs most affected are the tubules of the kidneys, blood vessels, heart, stomach and bronchi. There is evidence of irritation and degeneration in these tissues and in the liver. Under these conditions the animals lose weight rapidly, have intense diarrhea and die in from five to fourteen days. With smaller doses death comes later or the animal survives. Animals which live show the lesions described at least six months afterward. With diets which are deficient in either calcium or phosphorus, calcification is less severe and more delayed, but the degenerative changes are as severe as those found in animals fed a normal diet.

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[13] Bodansky, Aaron, and Jaffe, H. L.: Phosphatase Studies; Serum Phosphatase in Diseases of Bone; Interpretation and Significance, Arch. Int. Med. **54:** 88 (July) 1934. Morris, Noah; Stevenson, Mary M.; Pedan, Olive D., and Small, Jean M. D.: The Significance of Plasma Phosphatase in the Diagnosis and Prognosis of Rickets, Arch. Dis. Childhood **12:** 45 (Feb.) 1937.

[14] Shohl, A. T.; Goldblatt, Harry, and Brown, H. B.: The Pathological Effects upon Rats of Excess Irradiated Ergosterol, J. Clin. Investigation **8:** 505 (June) 1930. Harris.[6]

[15] Shohl, A. T., and Brown, H. B.: Rickets in Rats: XI. The Alteration of Calcium and Phosphorus Metabolism of Normal and Ricketic Rats Produced by Irradiated Ergosterol, J. Biol. Chem. **86:** 245 (March) 1930.

RELATION OF RICKETS TO GROWTH

Body growth and development are coordinated with, and limited by, skeletal growth. Paucity of minerals necessary for the formation of the bones retards not only the development of the skeleton but that of the entire body. Because bone salts are predominantly salts of calcium and phosphorus, it is not surprising that the ingestion, absorption, assimilation and utilization of these two elements are the principal factors which determine the development of the bone. Osborne and Mendel[16] showed that calcium or phosphorus could be the limiting factor of growth. Shohl and his collaborators[17] extended their studies to show that even with liberal additions of vitamin D to diets restricted in phosphorus, growth was limited to as much as 40 per cent of the normal.

It has long been a clinical dictum that the manifestations of rickets are proportional to the growth of the infant—"no growth, no rickets"—but from studies of experimental rickets it is obvious that the condition may be present without increase in weight or even with a decline in weight. In no case of experimental rickets is the growth as great as normal. With an otherwise adequate diet it is primarily the utilization of calcium and phosphorus which determines the rate of growth, and it is the interrelation of calcium and phosphorus and vitamin D which determines the development or the cure of rickets.

RELATION OF RICKETS TO DIET

The successful production of experimental rickets depends on the proper regulation of the calcium and phosphorus of the diet in the absence of vitamin D. In children and in some experimental animals, e. g., dogs,[18] a deficiency of vitamin D alone, without great distortion of the diet, is sufficient to produce rickets. With rats the calcium and phosphorus of the diet must be restricted or fed in unusual proportions. Complete reviews of the earlier literature may be found in the works of Goldblatt,[19] Hess,[2] György [2] and Gubner.[20] Briefly, it may be recalled that McCollum and his collaborators [21] investigated a wide variety of diets and obtained their best results with a diet of high calcium and low phosphorus content. They also reported success with low calcium and high phosphorus diets, although with these the bony lesions differed slightly from those which are found in children. When the calcium and phosphorus were about equal, they did not obtain rickets in rats. Sherman and Pappenheimer [22] and Steenbock and Black [23] also used high calcium and low phosphorus diets. With a low calcium and low phosphorus diet, Korenchevsky reported that rickets was not obtained, but the same diet with added calcium salts was shown by Goldblatt [24] to cause marked rickets. Thus it became a firmly fixed idea that the rachitogenic properties of a diet depend on the imbalance or disproportion between calcium and phosphorus. Experimental diets of this type, especially the Steenbock and Black No. 2965 (which is chemically similar in composition to the McCollum No. 3143), consist of 76 per cent corn, 20 per cent gluten flour, 3 per cent calcium carbonate and 1 per cent sodium chloride. The calcium content is about 1.2 per cent and the phosphorus about 0.25 per cent. The ratio of calcium to phosphorus is from about 4: 1 to 5: 1. The results obtained were so satisfactory that these diets became the standard rations for experimental production of rickets and also for the vitamin D assay. The necessity for constant and standard rachitic animals tended to discourage the study of other diets.

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[16] Osborne, T. B., and Mendel, L. B.: The Inorganic Elements in Nutrition, J. Biol. Chem. **34:** 131 (April) 1918.

[17] Shohl, A. T.; Brown, H. B.; Chapman, E. E.; Rose, C. S., and Saurwein, E. M.: The Evaluation of the Phosphorus Deficiency of the Rickets-Producing Diet, J. Nutrition **6:** 271 (May) 1933.

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[18] Mellanby, Edward: Experimental Rickets, Medical Research Council, Special Report Series, no. 61, London, His Majesty's Stat. Off., 1921. Shohl, A. T., and Bennett, H. B.: Rickets in Dogs: Metabolism of Calcium and Phosphorus, J. Biol. Chem. **76:** 633 (March) 1928.

[19] Goldblatt, Harry: Die neuere Richtung der Experimentellen Rachitisforschung, Ergebn. d. allg. Path. u. path. Anat. **25:** 58, 1931.

[20] Gubner, Richard: The Metabolic Background of Rickets, New England J. Med. **216:** 879 (May 20) 1937.

[21] McCollum, E. V.; Simmonds, N.; Shipley, P. G., and Park, E. A.: Studies on Experimental Rickets: VIII. The Production of Rickets by Diets Low in Phosphorus and Fat-Soluble A, J. Biol. Chem. **47:** 507 (Aug.) 1921.

[22] Sherman, H. C., and Pappenheimer, A. M.: Experimental Rickets in Rats: Diet Producing Rickets in White Rats, and Its Prevention by Addition of an Inorganic Salt, J. Exper. Med. **34:** 189 (Aug.) 1921.

[23] Steenbock, Harry, and Black, A.: Fat-Soluble Vitamins: XXIII. Induction of Growth-Promoting and Calcifying Properties in Fats and Their Unsaponifiable Constituents by Exposure to Light, J. Biol. Chem. **64:** 263 (June) 1925.

[24] Goldblatt, Harry: Experimental Rickets in Rats on a Purified Synthetic Diet Deficient in Phosphorus and Fat-Soluble Organic Factor, Biochem. J. **18:** 414 (no. 2) 1924.

More recently, the experimental diets used in the production of rickets were systematically investigated over a wide range of possible variations by Brown and others,[25] Querido [26] and Shohl.[1] This was made possible by the addition of varying amounts of salts of calcium and phosphorus to a basal diet of corn and gluten. The lower limit approximated calcium and phosphorus starvation, and the upper limit, which was obtained by feeding a diet which contained 20 per cent salt mixture, constituted all the calcium or the phosphorus that the animals would eat. It thus became obvious that not only the ratio of calcium to phosphorus but the absolute amount of each at that ratio determines the rachitogenic properties of the diet. As the ratio of calcium to phosphorus was increased for a given level of phosphorus, rickets became more intense (for low phosphorus diets). As the absolute amounts were diminished, the degree of rickets became more intense, not only for the high calcium–low phosphorus diets and the low calcium–high phosphorus diets but for the low calcium–low phosphorus diets. The last group of diets fell in the zone of calcium to phosphorus ratio of 2: 1 to 1: 2, which was previously considered to be "normal" or, at least, was not considered to be associated with rickets. In all cases in which the amounts of calcium and phosphorus were sufficient and the disproportion not too great, normal bones were produced and extra vitamin D was not needed.

Because both calcium and phosphorus are necessary to form bone, theoretically any substance which prevents the adequate utilization of either should lead to abnormalities in bones and possibly to rickets. Work on low calcium diets in this regard is lacking. With diets low in phosphorus, not only does a high calcium

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25. Brown, H. B.; Shohl, A. T.; Chapman, E. E.; Rose, C. S., and Saurwein, E. M.: Rickets in Rats: XIII. The Effect of Various Levels and Ratios of Calcium to Phosphorus in the Diet upon the Production of Rickets, J. Biol. Chem. **98:** 207 (Oct.) 1932.

26. Querido, Andries: Vitamin D Requirements in Relation to the Ca and P Content of the Diet, Arch. neérl. de physiol. **20:** 487, 1935.

level prevent adequate utilization of phosphorus but the other metals which form insoluble phosphates (except barium, which is too toxic even for experimental trial) cause rickets. In this list are beryllium,[27] magnesium,[28] strontium,[29] iron,[30] lead [31] and thallium.[32]

RICKETS AND THE ACID-BASE CONTENT OF THE DIET

The metabolism of vitamin D not only is interrelated with calcium and phosphorus but is dependent on the acidity of the diet. It has long been believed that acidity is associated with rickets and alkalinity with tetany. The acidity of the food can be considered both from the point of view of the true acidity, or hydrogen ion concentration, and from the stoichiometric point of view, in which consideration is given to the excess of mineral acids or bases. The true acidity exerts its effect principally on absorption from the intestinal tract. On the acid side the absorption of calcium and phosphorus is facilitated; on the alkaline side the absorption is retarded or inhibited. The potential acidity or alkalinity of a diet depends on the amount of alkaline elements, calcium, magnesium, sodium and potassium, as compared with the amount of acid elements, chlorine, sulfur and phosphorus. Acid foods, meaning those which have an excess of the acid elements, increase the excretion of calcium and phosphorus. Foods which are potentially alkaline tend toward diminished excretion and hence to a greater retention of calcium and phosphorus in the body. The total effect is therefore the resultant of the true and the potential acidity of the food.

An advance has recently been made by Hamilton and Schwartz,[33] who were able to separate and combine the effects of the diet on absorption and metabolism. By the use of a diet which provided an excess of organic acid and an alkaline ash, they facilitated both absorption and retention of calcium and phosphorus. Diets which were alkaline in reaction and which had an acid ash hindered both the absorption and the retention of calcium and phosphorus. These workers were able to convert rachitogenic diets into normal diets by the addition of organic acids and alkaline ash. They were able also to produce rickets by adding alkalis plus acid ash to normal diets. The former diets contained additions of tartaric acid and sodium tartrate, and the latter, additions of ammonium carbonate and ammonium chloride. Shohl[34] found that these effects were not due entirely to the acid-base properties of the diet but were due also to a specific organic acid effect, in which the tartrates were involved. The citrate ion showed even more pronounced effects. Additions of citric acid plus alkaline residue to rachitogenic diets were found to prevent or cure rickets. This result was obtained not with a single type of diet but with several widely different combinations of calcium and phosphorus.

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[27] Guyatt, B. L.; Kay, H. D., and Branion, H. D.: Beryllium "Rickets," J. Nutrition **6:** 313 (July) 1933.

[28] Park, E. A.: The Etiology of Rickets, Physiol. Rev. **3:** 106 (Jan.) 1923.

[29] Shipley, P. G.; Park, E. A.; McCollum, E. V.; Simmonds, N., and Kinney, E. M.: Studies on Experimental Rickets: XX. The Effects of Strontium Administration on the Histological Structure of the Growing Bones, Bull. Johns Hopkins Hosp. **33:** 216 (June) 1922.

[30] Diamond, L. K., and Brock, J. F.: Rickets in Rats by Iron Feeding, J. Pediat. **4:** 442 (April) 1934.

[31] Shelling, D. H.: Effect of Dietary Calcium and Phosphorus on Toxicity of Lead in the Rat: Rationale of Phosphate Therapy, Proc. Soc. Exper. Biol. & Med. **30:** 248 (Nov.) 1932.

[32] Rominger, E.; Meyer, Hugo, and Bomskov, C.: Rachitisstudien: die P-Stoffwechselstörung bei der experimentellen Thallium-Rachitis, Ztschr. f. d. ges. exper. Med. **78:** 272 (Aug.) 1931.

[33] Hamilton, Bengt, and Schwartz, Charles: Rôle of Acidosis and Alkalosis in the Etiology of Rickets, Am. J. Dis. Child. **46:** 669 (Sept.) 1933. Hamilton, Bengt, and Dewar, M. M.: Effect of Citrate and Tartrate on Experimental Rickets, Am. J. Dis. Child. **54:** 548 (Sept.) 1937.

UTILIZATION OF CALCIUM AND PHOSPHORUS

The diets which produce rickets exert a profound influence on the body economy. This is evidenced by their effect on the blood serum content of calcium and of inorganic phosphate. In general the composition of the blood serum in this respect reflects the composition of the intake. High calcium and low phosphorus rachitogenic diets are associated with a low inorganic phosphate content in the blood serum, a condition similar to that found in human rickets. Similarly, a low calcium and high phosphorus intake is associated with serum calcium values which are at or near the tetany level. In such cases the serum phosphorus is elevated roughly in proportion to the intake.

The absolute amounts as well as the ratio of the calcium to the phosphorus determine the content of these elements in the blood serum. At any ratio of calcium to phosphorus in the food, as the absolute amounts increase the serum calcium and phosphorus, respectively, increase also. When vitamin D is given, the blood serum values tend to become normal, regardless of the type of diet.[35]

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[34] Shohl, A. T.: The Effect of the Acid-Base Content of the Diet upon the Production and Cure of Rickets with Special Reference to Citrates, J. Nutrition **14**: 69 (July) 1937.

The effect of the acid-base content of the diet on the composition of the blood serum seems to be secondary to the ratio and level of calcium and phosphorus of the diet. In selected cases, however, it can be shown that acid ash diets produce a blood picture which tends toward rickets and that alkaline diets tend to cause healing.

It has been a favorite thesis of Freudenberg and György [36] that rickets is associated with an acid metabolism and tetany with an alkaline one. György has relied on determinations of the ammonia in the urine, a reliance which has been seriously questioned by Morris, Ford and Graham.[37] György also has cited early work of Hodgson,[38] who reported that greater doses of sodium bicarbonate were required to make the urine alkaline for rachitic children than for normal children. However, the classic rickets-producing diets are alkaline, and tetany may be induced even with acid phosphates. In neither clinical nor experimental rickets, nor in rachitic tetany, is there a definite alteration of the acid-base equilibrium of the blood.

After material has been absorbed from the intestine, it must take one of three paths. It may be subsequently excreted by the kidneys, it may be reexcreted into the intestine or it may be deposited in the tissues. Thus the amount of material in the feces represents both unabsorbed material and that which has been reexcreted through the intestine. Further, the calcium and phosphorus which are excreted may come either from that ingested or from that which is stored in the body. Calcium is excreted almost wholly by the bowel, about 90 per cent in infants and about 60 per cent in adults, and the major portion of the phosphorus may be excreted either by the kidneys or by the intestine. An excess of calcium causes a reduction in the urinary

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35. Park, E. A.; Guy, R. A., and Powers, G. F.: A Proof of the Regulatory Influence of Cod Liver Oil on Calcium and Phosphorus Metabolism, Am. J. Dis. Child. **26**: 103 (Aug.) 1923.

36. Freudenberg, E., and György, Paul: Zur Pathogenese der Tetanie, Jahrb. f. Kinderh. **96**: 5 (Oct.) 1921.

37. Morris, Noah; Ford, F. J., and Graham, Stanley: The Rôle of Acidosis and Phosphate Retention in Pathogenesis of Rickets and Rachitic Tetany of Infants, Acta paediat. **18**: 50 (Nov.) 1936.

38. Hodgson, Amy: Vitamin Deficiency and Factors in Metabolism Relative to Development of Rickets, Lancet **2**: 945 (Nov. 5) 1921.

phosphorus;[39] an excess of phosphorus results in low urinary calcium values.[40] Acid increases the urinary excretion of calcium and phosphorus,[41] and alkali increases the proportion found in the feces. Parathyroid hormone[42] also causes an increased excretion of calcium and phosphorus in the urine.

In clinical rickets there is an increased excretion of calcium in the feces, and the amount in the urine, normally small, is further decreased. The elimination of phosphorus in the feces is also markedly increased. As was first shown by Schabad,[43] it may exceed the fecal calcium. In infantile rickets both the calcium and the phosphorus balance are subnormal. They may be negative in severe rickets but usually are not.[44]

The effect of the administration of vitamin D is striking. Not only is the blood serum restored to its normal content of calcium and inorganic phosphate, but the metabolism is markedly altered. The amount of calcium and of phosphorus in the feces become reduced, while the amounts in the urine increase. The retention is also increased, so that one sometimes refers to the phenomenon as an increased "net absorption" of calcium and phosphorus.[6]

In experimental rickets the ratio of calcium to phosphorus retained reflects the amounts of calcium and phosphorus in the diet. When vitamin D is given, the blood serum values approach the normal. The absorption is affected similarly to that of infants, but the absolute retentions or the ratio of the calcium to the phosphorus retained may not be restored to normal. High calcium and low phosphorus diets result in high calcium and low phosphorus retentions, vitamin or no vitamin.[17]

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[39] Orr, W. J.; Holt, L. E., Jr.; Wilkins, L., and Boone, F. H.: The Relation of Calcium and Phosphorus in the Diet to the Absorption of These Elements from the Intestine, Am. J. Dis. Child. **28:** 574 (Nov.) 1924.

[40] Telfer, S. V.: Studies in Calcium and Phosphorus Metabolism: III. The Absorption of Calcium and Phosphorus and Their Fixation in the Skeleton, Quart. J. Med. **17:** 245 (April) 1924.

[41] Shohl, A. T., and Sato, A.: Acid-Base Metabolism: II. Mineral Metabolism, J. Biol. Chem. **58:** 257 (Nov.) 1923.

[42] Albright, Fuller; Bauer, Walter; Ropes, M., and Aub, J. C.: Studies of Calcium and Phosphorus Metabolism: IV. The Effect of the Parathyroid Hormone, J. Clin. Investigation **7:** 139 (April) 1929.

[43] Schabad, J. A.: Der Phosphorstoffwechsel bei Rachitis, Arch. f. Kinderh. **54:** 83, 1910.

[44] Bauer, Walter; Marble, Alexander, and Claflin, Dorothy: Studies on Mode of Action of Irradiated Ergosterol: Its Effect on Calcium, Phosphorus and Nitrogen Metabolism of Normal Individuals, J. Clin. Investigation **11:** 1 (Jan.) 1932. Nicolaysen, Ragnar: Studies on the Mode of Action of Vitamin D: II. The Influence of Vitamin D on the Fecal Output of Endogenous Calcium and Phosphorus in the Rat, Biochem. J. **31:** 107 (Jan.) 1937; III. The Influence of Vitamin D on the Absorption of Calcium and Phosphorus in the Rat, ibid. **31:** 122 (Jan.) 1937. Jones, J. H.; Rapoport, Milton, and Hodes, H. L.: The Effect of Irradiated Ergosterol on the Absorption of Calcium from the Gastro-Intestinal Tract, Am. J. M. Sc. **179:** 729 (May) 1930. McGowan, J. P.: Further Investigations into Fundamental Nature of Vitamin D Action, Biochem. J. **27:** 943 (no. 3) 1933. Rominger, E.; Meyer, H., and Bomskov, C.: Bemerkungen zu der Arbeit "Experimentelle Rachitis": II. Der Calcium- und Phosphorstoffwechsel bei der Entwicklung und Heilung der Rachitis von Dr. H. Stolzberg und Dr. G. Meyer zu Hörste, Monatschr. f. Kinderh. **55:** 206 (Nov.) 1932.

Vitamin D and Parathyroid.—The relationship of vitamin D and parathyroid hormone has been intensively investigated.[45] It was suspected that their actions were interdependent, but it now seems clear that, although both raise the level of calcium and of phosphorus, the action of each is quite different. Parathyroid acts more specifically on the serum calcium. When it is given for parathyroprivic tetany the serum calcium level is dramatically raised and the phosphate content may be depressed. Only when toxic doses are given is the effect in raising the phosphate level marked. Vitamin D has its most spectacular action in raising a low serum phosphate level in rickets. As previously stated, in toxic doses it increases the calcium content above normal and may depress the phosphate content if it is high. Parathyroid seems to act principally by withdrawal of calcium from the body; vitamin D, by increased absorption, or diminished reexcretion into the bowel. The toxic effect of parathyroid is decalcification; that of vitamin D, hypercalcification.

In rickets, as originally shown by Erdheim and repeatedly confirmed, the parathyroid glands enlarge and the cells hypertrophy. This occurs in human beings, rats, rabbits and fowl. Hamilton and Schwartz [46] were able to demonstrate an increased hormone content in the serum of rachitic rabbits. This overactivity of the parathyroid glands is not the cause of rickets but is caused by rickets. The reaction is apparently a defense mechanism of the body to preserve the normal level of serum calcium, so essential to the body economy. As far as rickets is concerned, this hyperfunction of the parathyroids intensifies rather than ameliorates the condition. Shelling, Asher and Jackson,[47] Collip and his co-workers [45] and Waltner [48] found that injection of the hormone exaggerated the rachitic process in rats. Hoag and his associates [49] and Fine and Brown [50] found that it retarded the healing. Such action depends on the type of diet. With a normal diet, not rickets but osteitis fibrosa cystica results. Further, Pappenheimer [51] and Shelling [52] both stated that it is more difficult to produce rickets after removal of the parathyroids.

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[45] Collip, J. B.; Pugsley, L. I.; Selye, H., and Thomson, D. L.: Observations Concerning the Mechanism of Parathyroid Hormone Action, Brit. J. Exper. Path. **15**: 335 (Dec.) 1934.

[46] Hamilton, Bengt, and Schwartz, Charles: Rickets and Hyperparathyroidism, Am. J. Dis. Child. **46**: 775 (Oct.) 1933.

OTHER PHASES OF VITAMIN D METABOLISM

The metabolism of calcium and phosphorus has been discussed in relation to vitamin D. Certainly the metabolism of magnesium and of iron also must be affected. The relation to carbohydrate metabolism has been hinted at in relation to phosphatase. The fat metabolism also is interrelated to the problem. If fat is not utilized, the fatty acids are excreted in the intestine, where they form calcium soaps and thus interfere with the calcium and phosphorus economy.[53] In such conditions as fatty diarrhea and celiac disease, rickets may supervene. Rickets thus caused is amenable to vitamin D therapy.

A great deal has been written on the relation of dental caries to vitamin D, especially by Mellanby [54] and the English workers. Insofar as the teeth are part of the bony structure, the relation to vitamin D seems straightforward. However, the explanation of the underlying physiologic and pathologic process is still clouded, and the multiplicity of therapies indicates that vitamin D is not the sole factor in caries prevention.

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47. Shelling, D. H.; Asher, D. E., and Jackson, D. A.: Calcium and Phosphorus Studies: Effects of Variations in Dosage of Parathormone and of Calcium and Phosphorus in Diets on Concentrations of Calcium and Inorganic Phosphorus in Serum and on Histology and Chemical Composition of Bones of Rats, Bull. Johns Hopkins Hosp. **53:** 348 (Dec.) 1933.

48. Waltner, Karl: Ueber die Funktion der Nebenschilddrüse, Monatschr. f. Kinderh. **40:** 317, 1928.

49. Hoag, L. A.; Rivkin, Helen; Weigele, C. E., and Berliner, Frieda: Effect of Potent Parathyroid Extract on Calcium Balance in Infants, Am. J. Dis. Child. **33:** 910 (June) 1927.

50. Fine, J., and Brown, S.: Influence of Parathormone on Bone Regeneration, New England J. Med. **198:** 932 (June 21) 1928.

51. Pappenheimer, A. M.: Antirachitic Action of Cod Liver Oil and Irradiated Ergosterol in Parathyroidectomized and Thymectomized Rats, J. Exper. Med. **52:** 805 (Dec.) 1930.

52. Shelling, David H.: The Parathyroids in Health and in Disease, St. Louis. C. V. Mosby Company, 1935.

53. Telfer, S. V.: Studies in Calcium and Phosphorus Metabolism: IV. The Influence of Free Fatty Acids in the Intestine on Absorption and Excretion of the Mineral Elements, Quart. J. Med. **20:** 1 (Oct.) 1926.

54. Mellanby, May: The Influence of Diet on the Structure of Teeth, Physiol. Rev. **8:** 545 (Oct.) 1928.

For the pregnant or lactating woman faulty diet and paucity of vitamin D present a real problem. There is no doubt that the offspring may come into the world with poorer reserves of antirachitic factors. The mother and infant show symptoms of calcium and phosphorus deficiency.[55] Congenital tetany and early rickets are found when searched for. The fact that vitamin D is excreted in the milk[56] suggests the possibility of prophylaxis against rickets in the infant through ingestion of vitamin D by the mother.

Renal rickets is associated with damaged kidney and upset of the phosphate excretion, often complicated by acidosis.[57]

Finally, intractable rickets has been reported, for which the therapeutic effect of vitamin D could be obtained only when the dose was multiplied many thousand times. These are but a few of the problems the solution of which lies in the future.

SUMMARY

To summarize known facts, it may be stated that the study of the physiology and pathology of vitamin D has advanced to the point where it is recognized that:

The metabolism of vitamin D is closely related to the composition of the diet, especially the content of calcium and of phosphorus and the acid-base value.

The metabolism of vitamin D is related to the parathyroid hormone, phosphatase and other factors.

The main action of vitamin D is to increase the absorption of calcium and phosphorus or to diminish their intestinal excretion.

When the concentration of Ca^{++} and $(PO_4)^{---}$ in the body fluid surrounding the degenerating cartilage cell as measured by the state in the blood is sufficiently great, deposition of salts in growing bone occurs.

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[55] Maslow, H. L.: Tetany in Newborn, Arch. Pediat. **50**: 768 (Nov.) 1933.

[56] Light, R. F.; Wilson, L. T., and Frey, C. N.: Vitamin D in the Blood and Milk of Cows Fed Irradiated Yeast, J. Nutrition **8**: 105 (July) 1934.

[57] Mitchell, A. G.: A Consideration of the Pathogenesis of Renal Rickets, Acta paediat. **11**: 352, 1930.

CHAPTER XXV

THE DETERMINATION AND SOURCES OF VITAMIN D

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The observations that rickets can be produced in rats [1] and that healing of induced rickets occurs in the proliferative zone of the cartilage led to the development of quantitative methods for the determination of vitamin D by the so-called line test procedure.[2] The basis for this procedure is the fact that within certain limits and under properly controlled conditions the degree of healing induced is proportional to the quantity of vitamin D fed. Severe rickets will develop in young rats in about three weeks, and curative periods of from five to ten days are sufficient to produce healing which can be evaluated. The degree of healing can be determined roentgenographically, but the most common procedure is to kill the animal and section and stain one of the leg bones.

Prior to the adoption of the U. S. P. method in 1934 the vitamin D content of pharmaceutical products in this country was indicated in three different units (Steenbock, A. D. M. A. and Poulsson, or Oslo), whose relative value to one another could only be approximated. Each one of these units was defined in terms of a method of assay bearing the same name. Fundamentally the procedures were the same and they were about equally serviceable, but the use of units of different values was responsible for confusion and misunderstanding. The elimination of this confusion was accomplished through the adoption in 1931 by the

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From the the Food and Drug Administration.

[1] Sherman, H. C., and Pappenheimer, A. M.: A Dietetic Production of Rickets in Rats and Its Prevention by an Inorganic Salt, Proc. Soc. Exper. Biol & Med. **18:** 193-197 (March 9) 1921. McCollum, E. V.: Simmonds, Nina; Parsons, H. T.; Shipley, P. G., and Park, E. A.: Studies on Experimental Rickets: I. The Production of Rachitis and Similar Diseases in the Rat by Deficient Diets, J. Biol. Chem. **45:** 333-341 (Jan.) 1921.

[2] McCollum, E. V.; Simmonds, Nina; Shipley, P. G., and Park, E. A.: Studies on Experimental Rickets: XVI. A Delicate Biological Test for Calcium-Depositing Substances, J. Biol. Chem. **51:** 41-49 (March) 1922.

Health Organization of the League of Nations of an International standard for vitamin D[3] and an International unit, which was defined in terms of a definite quantity of the standard. A similar vitamin D standard had been used in Great Britain prior to that time. The International standard for vitamin D is irradiated ergosterol, prepared under specified conditions, dissolved in olive oil. The International unit is defined as "the vitamin D activity of 1 milligram of the International standard solution of irradiated ergosterol, which has been found equal to that of 0.025 microgram of crystalline vitamin D."

The International standards for vitamins are prepared under the auspices of the Health Organization of the League of Nations, and allotments are sent without charge to a central distributing agency in each country. With the extensive commerce in vitamin preparations in this country and consequent heavy demands for standards, it seemed desirable to conserve the supply of International standards. Accordingly the United States Pharmacopeia adopted (1934 Interim Revision) as a standard for vitamins A and D a specimen of cod liver oil and designated it the U. S. P. (Pharmacopeia) Reference Cod Liver Oil. A suitable quantity of a specially prepared cod liver oil was put up in ounce bottles. The oil was then assayed against the International standards for vitamins A and D. A U. S. P. unit of vitamin D is defined as "equal, in antirachitic potency for the rat, to one International Unit of Vitamin D as defined and adopted by the Conference of Vitamin Standards of the Permanent Commission on Biological Standardization of the League of Nations in June of 1931."

The method of assay referred to is the same as that given in the current Pharmacopeia and, while specific for cod liver oil, with slight modifications is suitable for the assay of a wide variety of vitamin D preparations. The method provides for comparing the vitamin D content of the oil under assay with the U. S. P. Reference Cod Liver Oil by the line test procedure and specifies the conditions for raising the experimental animals, the limits of the period for producing rickets, the duration of the assay period, the number of animals and their age and weight limits, feeding methods, a method for evaluating the degree of healing, the data that must be recorded, and how the vitamin D potency of the oil shall be calculated.

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[3] Report of the Conference on Vitamin Standards, Geneva, Publications Department of the League of Nations, No. C.H. 1055 (1), 1931.

In applying this method to various types of products, due recognition must be given to its limitations. Success in developing experimental rickets is due in part to the feeding of a high proportion of calcium as compared to phosphorus. A disturbance of the prescribed calcium-phosphorus ratio will influence the degree of healing. The rate of healing is also influenced by change in the acid-base balance of the diet, ingestion of products which affect the rate of growth or compounds which influence the assimilation of calcium or phosphorus. For these reasons it is difficult and sometimes impossible to obtain satisfactory assays on food products by direct feeding. With foods fortified with vitamin D these problems can usually be met by extraction of the vitamin with suitable solvents or modification of the basal diet of the control animals.

While the U. S. P. or similar methods involving the line test are most extensively used for vitamin D determinations, the percentage of ash in the fat-free leg bones of the rat may also be used as a criterion in vitamin D assays. This procedure was used by Bethke, Steenbock and Nelson[4] as early as 1923 but has never received wide acceptance. It is more tedious than the line test but, being objective, could probably be developed to a greater degree of precision. However, efforts in this direction have not produced particularly promising results. Bone ash is also used as a criterion in vitamin D assays, with baby chicks in the tentative A. O. A. C. method.[5] The fact that rats and chicks do not respond in the same manner to different types of vitamin D[6] makes it possible by duplicate tests with rats and chicks to differentiate between vitamin D from irradiated ergosterol and from cod liver oil. While 20 U. S. P. units of vitamin D from cod liver oil per hundred grams of feed will permit development of chicks with normal bone ash, from 500 to 2,500 units of vitamin D in the form of activated ergosterol per hundred grams of feed may be required to provide the same degree of protection. The ratio widens with lengthening of the test period.

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[4] Bethke, R. M.; Steenbock, Harry, and Nelson, M. T.: Fat Soluble Vitamins: XV Calcium and Phosphorus Relations to Growth and Composition of the Blood and Bone with Varying Vitamin Intake, J. Biol. Chem. **58:** 71-103 (Nov.) 1923.

[5] Vitamin D Assay by Preventive Biological Test, J. Off. Agric. Chem. **20:** 72-73 (Feb.) 1937.

[6] Bills, C. E.: Physiology of the Sterols, Including Vitamin D, Physiol. Rev. **15:** 1-97 (Jan.) 1935.

Biologic methods for some of the other vitamins are being supplemented by physical and chemical methods, but the latter are of practically no value in the determination of vitamin D. They can of course be applied to preparations of a high degree of purity and therefore have application in certain manufacturing processes. Evidence is accumulating that there are several forms of naturally occurring vitamin D and that their physiologic properties may differ. Vitamin D is a substance having great physiologic activity. A barrel of average grade cod liver oil contains approximately 0.25 Gm. of vitamin D. Pure vitamin D made by irradiation of ergosterol contains forty million units of vitamin D per gram. The chemist needs only one millionth of a gram for a biologic assay. Owing to the small quantities of vitamin D present in products, chemical tests are practically limited to color tests. When one considers that vitamin D belongs to the ubiquitous sterols, the probability of developing color tests with a high degree of specificity seems rather remote.

FOOD SOURCES OF VITAMIN D

There appears to be no proof that vitamin D exists in living plant tissue, and fresh green vegetables are generally considered entirely devoid of this vitamin. Plant tissue which is no longer living will, at least in some instances, acquire significant vitamin D potency when exposed to the sun, as is evidenced by the presence of vitamin D in hays [7] and cacao shells.[8] Natural foods which contain vitamin D are of animal origin.[9] Fish which contain much body oil, such as salmon, sardines and herring, are the richest natural sources; eggs are next in importance, and milk fat and meat products contain some vitamin D. Since this vitamin is not found in appreciable quantities in so many of the foods ordinarily consumed and since evidence of acute vitamin D deficiency occurs only in infants, it appears that the vitamin D requirement of man is very low or that his needs are usually provided for by exposure to sunshine.

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[7] Steenbock, Harry; Hart, E. B.; Elvehjem, C. A., and Kletzien, S. W. F.: Dietary Factors Influencing Calcium Assimilation: VI. The Antirachitic Properties of Hays as Related to Climatic Conditions with Some Observations on the Effect of Irradiation with Ultraviolet Light, J. Biol. Chem. **66**: 425-440 (Dec.) 1925.

[8] Knapp, A. W., and Coward, K. H.: Vitamin D in Cacao Shells, Analyst **59**: 474-478 (June) 1934.

[9] Compilations are given in Miscellaneous Circular 275, United States Department of Agriculture, June 1937.

Of the common foods fortified with vitamin D, only milk needs to receive serious consideration. The remainder are all too frequently transients and usually emblazoned with statements of their alleged virtues in a manner that cannot escape notice. If the use of foods fortified with vitamin D is contemplated, the labels of the products should be examined to determine whether their vitamin content is stated in units so that the claims can be checked. The types of vitamin D milk that are acceptable to the Council on Foods and the allowable claims for such milk have been published in THE JOURNAL.[10] Approximately one half of the evaporated milk manufactured is fortified with vitamin D, and approximately 700 dairies sell milk similarly fortified.

PHARMACEUTICAL PREPARATIONS

The vitamin D of pharmaceutical products is derived either from natural fish liver oils or artificially produced activated sterols. Some of the fish liver oils are offered as the pure oil, such as cod liver oil and halibut liver oil, and viosterol in oil contains only activated ergosterol in an edible vegetable oil, but a great many of the products offered are combinations of several of the sources of vitamin D. In addition the vitamin D preparations take the form of emulsions, tablets, capsules and malt preparations. The fact that the vitamins of fish liver oils can be removed in the unsaponifiable fraction, concentrated by direct extraction of the oil, or even distilled from the oil under a high vacuum makes it possible to manufacture preparations which have advantages in ease of administration. Products which are either tasteless or very palatable are readily available.

With an ever increasing array of vitamin D preparations before him, what guide does the physician have in the selection of a vitamin D preparation with respect to vitamin D content? There are U. S. P. standards for only three vitamin D preparations. "Cod Liver Oil" is required by the U. S. Pharmacopeia to contain at least 85 U. S. P. units of vitamin D per gram. A teaspoonful contains at least 312 units, calculated on the basis that activated ergosterol per hundred grams of feed may be required to provide the same degree of protection. The ratio widens with lengthening of the test period.

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[10] The Present Status of Vitamin D Milk, Report of the Council on Foods, J. A. M. A. **108**: 206-207 (Jan. 16) 1937.

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[7] Steenbock, Harry; Hart, E. B.; Elvehjem, C. A., and Kletzien, S. W. F.: Dietary Factors Influencing Calcium Assimilation: VI. The Antirachitic Properties of Hays as Related to Climatic Conditions with Some Observations on the Effect of Irradiation with Ultraviolet Light, J. Biol. Chem. **66**: 425-440 (Dec.) 1925.

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[10] The Present Status of Vitamin D Milk, Report of the Council on Foods, J. A. M. A. **108:** 206-207 (Jan. 16) 1937.

During the past few years a large number of new types of vitamin D preparations have appeared on the market. With the discovery of new sources of vitamin D and new methods of preparing vitamin concentrates and the application of new scientific methods in the synthesis of the vitamin, more types of products must be expected before the industry becomes stabilized.

SUMMARY

Within the past few years the method of expressing vitamin D potency has become standardized. Vitamin D content is expressed either in U. S. P. or International units, and these units are equal in value. Biologic methods for the determination of vitamin D are now available which permit satisfactory control over preparations intended for use by the physician. There

are at present no physical or chemical methods which give promise of replacing biologic assays.

Most foods appear to be devoid of demonstrable quantities of vitamin D. Fish which contain much body oil, such as salmon, sardines and herring, are the richest natural sources, eggs are next in importance, and milk fat and meat products contain some vitamin D. Vitamin D milk is now being used extensively as a dependable dietary source of vitamin D.

A great variety of pharmaceutical preparations of vitamin D are available for therapeutic use, and new types can be expected to make their appearance. Since there are minimum standards for vitamin D content for only a few of these preparations, statements of vitamin potency must be relied on for their intelligent use.

CHAPTER XXVI

THE HUMAN REQUIREMENT OF VITAMIN D

P. C. JEANS, M.D.
AND
GENEVIEVE STEARNS, PH.D.
IOWA CITY

For many persons some vitamin D in addition to that ordinarily obtained by exposure to sunshine is necessary for the most efficient utilization of calcium and phosphorus. The vitamin acts by increasing the amounts of these substances available for the mineralization of bones and teeth. Measurement of the requirement of vitamin D presupposes the ingestion of a diet adequate in all other respects and particularly containing ample amounts of calcium and phosphorus; it should include also recognition that the human requirement of vitamin D (or of any other nutritional factor) includes both the quantity necessary to prevent obvious clinical pathologic changes and an amount which promotes a condition of normal nutrition and health.

The requirements of vitamin D may be defined as those amounts which, with ample intakes of calcium and phosphorus and a diet otherwise adequate, insure sufficient retention of calcium and phosphorus to permit (*a*) normal growth and mineralization of the skeleton and teeth of infants and children, (*b*) maintenance of bony and dental structures during adult life and (*c*) a sufficient supply for mother and infant during pregnancy and lactation.

Unfortunately no one yet knows the normal rate of growth of children. Instead, there are available only many average rates of growth under widely varying conditions of nutrition. It is known from studies of racial food habits that when the diet of a people is nutritionally good and includes a plethora of milk and ingested vitamin D or its equivalent in sunlight the

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From the Department of Pediatrics, State University of Iowa College of Medicine.

people of that race are tall and well formed, with well calcified skeletons and freedom from dental caries.[1] On the other hand, peoples whose mineral or vitamin D intake is very low are small of stature and prone to be subject to rickets or osteomalacia.[2] Even in this country, children from better class homes are taller and heavier than children of poor families,[3] and children from better class homes of today are taller than those of the same class a generation or so ago.[4] One may postulate, therefore, that "average" rates of growth of today still represent suboptimal rather than optimal skeletal growth, so that, if linear growth is used as a criterion of adequacy of vitamin D intake, the rate should be at least "average."

During childhood and adolescence, not only does the skeleton grow in size but a part of its water content is replaced by inorganic salts. The rate of replacement is not known with certainty but is most rapid in early life. Because of the variability of standards of growth and mineralization of the skeleton, the optimal amounts of calcium and phosphorus to be retained at any age are not known. Various methods of calculating these requirements have appeared in the literature from time to time.[5] The required retentions estimated for calcium by the different investigators vary from 0.1 Gm. daily throughout childhood[5a] to a graded scale increasing from 0.3 Gm. daily at 1 year to 0.5 Gm. daily during adolescence.[5c] As improvement in nutrition has so far resulted in increased average stature, and as mineralization of the skeleton may lag far behind growth,[6] the higher figures are thought to be a safer guide.

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[1] McCarrison, R.: Problems of Nutrition in India, Nutrition Abstr. & Rev. **2:** 1 (July) 1932. Orr, J. B., and Gilkes, J. L.: Physique and Health of Two African Tribes, Medical Research Council, Special Report Series, No. 155, London, His Majesty's Stationery Office, 1931. Ehrström, R.: Die Diät- und Kostführung der nordischen Länder in historischer Beleuchtung, Acta med. Scandinav. **81:** 583 (fasc. V-VI) 1934.

[2] Maxwell, J. P.: Osteomalacia and Diet, Nutrition Abstr. & Rev. **4:** 1 (July) 1934. Footnote 1.

[3] Baldwin, B. T.: Physical Growth of Children from Birth to Maturity, University of Iowa Studies, Studies in Child Welfare I, No. 1, June 1921. Gray, Horace, and Fraley, Frederick: Growth Standards: Height, Chest-Girth and Weight for Private School Boys, Am. J. Dis. Child. **32:** 554 (Oct.) 1926.

[4] Gray, Horace: Increase in Stature of American Boys in the Last Fifty Years, J. A. M. A. **88:** 908 (March 19) 1927.

[5] (*a*) White House Conference Reports: Growth and Development of the Child: III. Nutrition, New York, the Century Company, 1932, p. 196. (*b*) Sherman, H. C., and Hawley, E.: Calcium and Phosphorus Metabolism in Childhood, J. Biol. Chem. **53:** 375 (Aug.) 1922. (*c*) Leitch, I.: The Determination of the Calcium Requirements of Man, Nutrition Abstr. & Rev. **6:** 553 (Jan.) 1937.

The requirements of normal persons for only the ingested forms of vitamin D will be considered in the present discussion. The criteria commonly used are freedom from rickets or osteoporosis, good growth and development, normal values for serum calcium and phosphorus, ample retentions of these elements for growing individuals and "equilibrium" for those fully grown.

REQUIREMENT DURING INFANCY

Infants Fed Cow's Milk.—Studies of the vitamin D requirement during infancy may be divided into two groups. The larger group comprises studies planned to determine a minimal requirement using the prevention of rickets as the sole or chief criterion. Some of these include also the levels of serum calcium, phosphorus and phosphatase, and a few have taken into consideration the rates of growth. The smaller group comprises studies which include in addition to the preceding criteria quantitative determinations of the retention of calcium and phosphorus correlated with the rate of growth and the relative intakes of calcium and phosphorus. The emphasis in this group has been not on rickets prevention but on growth and development and on the fully adequate rather than the minimum requirement for vitamin D.

All these studies include periodic roentgen examination of the radius or tibia; in the first group of studies this constitutes the chief basis of judgment. Little agreement has been achieved in the interpretation of minor aberrations of growth at the epiphysial line,[7] but much greater uniformity in the determination of rickets of "clinical significance." Use of only the latter criterion is necessary if comparisons are to be made between the various studies. Rickets prevention alone probably should not be considered as a criterion of an adequate vitamin D supply. It is at least reasonable to think that the optimum amount of any nutritional essential is somewhat greater than the amount which barely prevents clinical pathologic changes.

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[6] (*a*) Todd, T. W.: Personal communication to the authors. (*b*) Sherman, H. C., and Campbell, H. L.: Growth and Reproduction on Simplified Food Supply: IV. Improvement in Nutrition Resulting from an Increased Proportion of Milk in the Diet, J. Biol. Chem. **60:** 5 (May) 1924.

[7] (*a*) Jeans, P. C.: Vitamin D Milk, J. A. M. A. **106:** 2066 (June 13), 2150 (June 20) 1936. (*b*) Cooley, T. B., and Reynolds, Lawrence: The Interpretation of X-Ray Films in the Diagnosis of Rickets, J. Pediat. **10:** 743 (June) 1937.

The determination of calcium and inorganic phosphorus of blood serum has a definite but limited usefulness in estimating the vitamin D requirement. In general, rickets cannot develop concurrently with a continuously normal level. The amounts of vitamin D that barely prevent rickets produce serum values within the range considered normal. However, values of this magnitude give little indication as to whether the calcium balance is weakly or strongly positive; it may even be negative. Perhaps one should distinguish between low and high normal levels. When the amount of vitamin D is considered fully adequate, the serum calcium and phosphorus levels then would be in the upper part of the normal range, whereas with amounts of vitamin D considered inadequate the serum values would be at the lower limits of normal.

The rate of growth is an important factor in determining the vitamin D requirement. Since rickets is a condition the severity of which tends to increase with increasing rates of growth, it is more important to prove that rickets has been prevented in a rapidly growing than in a slowly growing infant. Evidence is available also that the rate of growth is increased above the average when the intake of vitamin D is fully adequate.[8]

The most useful criterion of the vitamin D requirements is provided by studies of the utilization of calcium and phosphorus. Observations on the retention of these minerals permit a direct evaluation of the efficiency of utilization at different levels of vitamin D intake instead of the indirect estimates which have been discussed. This direct evaluation is the one procedure available which permits the estimation of an appropriate rather than a minimal requirement for vitamin D. Rickets prevention can be a criterion only in infancy, whereas the retained quantity of calcium and phosphorus offers a criterion for all age periods. Ample rather than minimal retention of calcium and phosphorus is required throughout the period of growth to supply material for new bone and for the replacement of water in bone already formed. Ample retention of these elements is desirable also to aid in compensating for the inevitable mineral losses in association with illness.

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[8] (*a*) Stearns, Genevieve; Jeans, P. C., and Vandecar, Verva: The Effect of Vitamin D on Linear Growth in Infancy, J. Pediat. **9:** 1 (July) 1936. (*b*) Slyker, F.; Hamil, B. M.; Poole, M. W.; Cooley, T. B., and Macy, Icie G.: Relationship Between Vitamin D Intake and Linear Growth in Infants, Proc. Soc. Exper. Biol. & Med. **37:** 499 (Dec.) 1937.

The consensus of various studies seems to be that the quantity of vitamin D received by a baby fed customary quantities of milk containing 135 units[9] to the quart is sufficient to prevent the development of rickets in the majority of full term infants but insufficient to prevent moderate or severe rickets in prematurely born infants; that from 300 to 400 units daily as cod liver oil or as milk containing 400 units to the quart allows sufficient vitamin D to prevent rickets of clinical significance in both full term and prematurely born infants.[10]

Early studies reported different normal standards for the serum calcium and phosphorus levels of breast-fed and artificially fed babies. Later studies showed that artificially fed infants given adequate vitamin D had serum values equaling those of infants given human milk.[11] When infants are fed milk containing 135 units of vitamin D to the quart, the serum calcium and phosphorus values are normal, but higher dosages of vitamin D (from 300 to 400 units) permit levels nearer the top of the normal range.[12]

It has been observed that infants given milk containing 135 units to the quart tend to grow at average rates, whereas infants given from 300 to 400 units of vitamin D tend to grow somewhat faster than the average.[8a] This observation has been corroborated recently.[8b]

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[9] All units mentioned in this review refer to U. S. P. XI units of vitamin D.

[10] (*a*) Rapoport, Milton, and Stokes, Joseph, Jr.: II. The Antirachitic Value of Irradiated Evaporated Milk and Irradiated Whole Fluid Milk in Infants, J. Pediat. **8:** 154 (Feb.) 1936. (*b*) Eliot, Martha M.; Nelson, E. M.; Barnes, D. J.; Browne, Florence A., and Jenss, Rachel M.: A Study of the Comparative Value of Cod Liver Oil, Viosterol and Vitamin D Milks in the Prevention of Rickets and of Certain Basic Factors Influencing their Efficacy, ibid. **9:** 355 (Sept.) 1936. (*c*) Drake, T. G. H.; Tisdall, F. F., and Brown, Alan: Irradiated Evaporated Milk in the Prevention of Rickets, ibid. **8:** 161 (Feb.) 1936; A Comparison of the Antirachitic Effect of Irradiated Cholesterol and Cod Liver Oil, ibid. **9:** 421 (Oct.) 1936. (*d*) Davidson, L. T.; Merritt, Katharine K., and Chipman, S. S.: Prophylaxis of Rickets in Infants with Irradiated Evaporated Milk, Am. J. Dis. Child. **53:** 1 (Jan., pt. 1) 1937; (*e*) Prophylaxis of Rickets in Infants with Vitamin D Milk, ibid. **51:** 1 (Jan.) 1936. Jeans.[7a]

[11] Stearns, Genevieve, and Warweg, Edna: Studies of Phosphorus of Blood: I. The Partition of Phosphorus in Whole Blood and Serum, the Serum Calcium and Plasma Phosphatase from Birth to Maturity, J. Biol. Chem. **102:** 749 (Oct.) 1933.

[12] (*a*) Jeans, P. C., and Stearns, Genevieve: The Retention of Calcium by Infants Fed Evaporated Milk Containing Cod Liver Oil Concentrate, Proc. Soc. Exper. Biol. & Med. **32:** 1468 (June) 1935. (b) McQuarrie, Irvine; Thompson, W. H.; Stoesser, A. V., and Rigler, L. G.: The Antirachitic Potency of Ergosterol Activated by Low Velocity Electrons, J. Pediat. **10:** 295 (March) 1937.

Studies of calcium and phosphorus retention[13] have shown that infants given no additional vitamin D show marked variability in calcium retention with a low average. When from 60 to 135 units of vitamin D is given daily, the number of low retentions decreases, raising the average retention of the group. A further and significantly greater increase in average retention is obtained (by raising the lower limit of the retention range) when from 300 to 400 units of vitamin D is fed daily. Thus, increasing the vitamin D content of the diet from 0 to 300 or 400 units decreases variability of the infant in regard to ability to retain calcium and tends to raise all infants to the level of those most

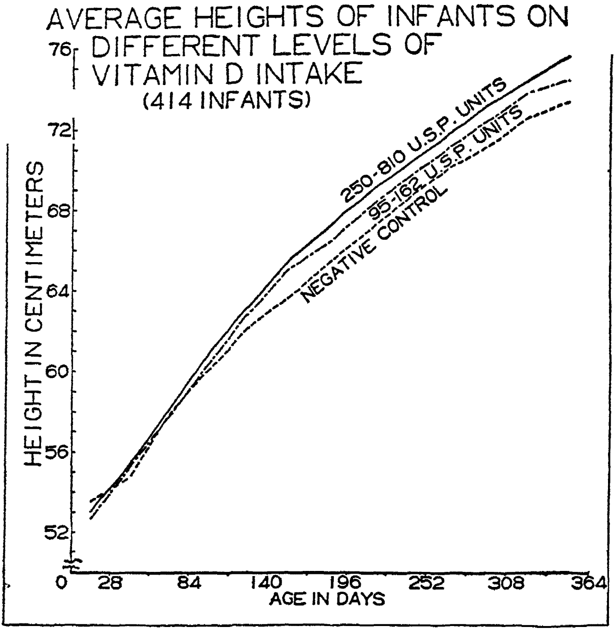

Chart 1.—The effect of vitamin D on linear growth in infancy. The infants of the upper group received an average of 600 to 675 units of vitamin D daily. Reproduced by permission of the authors and the Proceedings of the Society for Experimental Biology and Medicine.[8b]

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[13] Stearns, Genevieve, and Jeans, P. C.: The Effect of the Quantity of Vitamin D Intake on Calcium Retention in Infancy, J. Biol. Chem. **114:** c (May) 1936.

efficient in calcium retention. These studies show also that the calcium retentions observed when from 300 to 400 units of vitamin D is fed daily are accompanied by excellent dentition and the maintenance of serum calcium and phosphorus at high normal levels as well as by increased growth and development. The average retention observed when from 300 to 400 units of vitamin D is given apparently is ample to provide

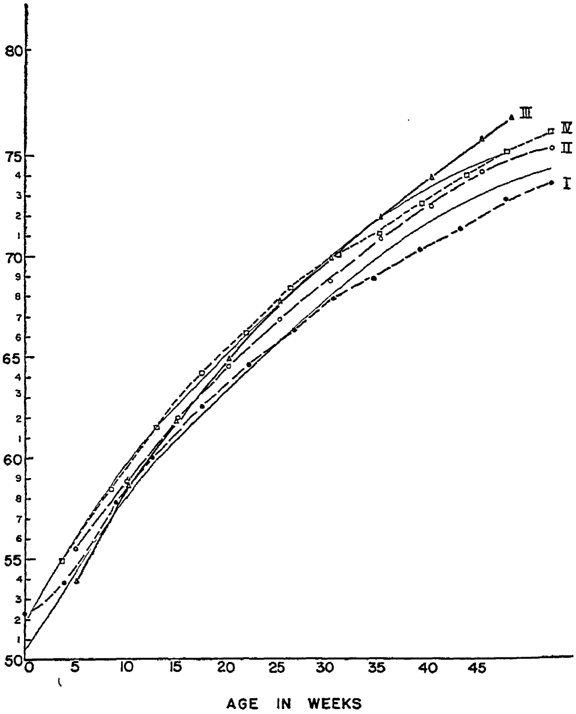

Chart 2.—The effect of excessive vitamin D intake on linear growth in infancy. The data are taken from Iowa studies[8a, 14] and from Peatman and Higgons.[14a] The basal diets of all groups were similar. The fine lines represent average rates of growth of male infants (Kornfeld). The curves show the rates of linear growth of male infants given the following amounts of vitamin D. I, no vitamin D ingested (Baldwin cf.[8a]); II, milk containing 135 units to the quart; III, 340 units as cod liver oil; IV, 1500-9000 units daily.[14a] Between the ages of 8 and 48 weeks the infants given 340 units of D daily grew 3 cm. more than those given 1500 or more units, 3¼ cm. more than those given 135 unit milk and 4 cm. more than those given no vitamin D. The effect of vitamin D on increasing linear growth in infancy appears to reach a maximum, and marked increases in vitamin D intake apparently result in slowing of growth after the age of 6 months.[14]

abundantly the nutritional needs for these minerals, even at the somewhat increased rate of skeletal growth observed.

Data are not available to show whether an amount of vitamin D between 135 and 300 units daily would be equally effective in permitting ample retention of calcium and phosphorus. Whatever the minimal level of vitamin D for good retention of these minerals, that level seems to be greater than 135 units.

A few observations indicate that amounts of vitamin D which are considerably in excess of 400 units daily may be detrimental when growth and retention are used as criteria. In an inpatient study,[14] nine infants given 1,800 or more units of vitamin D daily showed rates of linear growth similar to, or less than, those of infants given a similar dietary regimen but receiving 135 unit vitamin D milk. The rates of growth were definitely lower than those of infants given 340 units of vitamin D. Decreasing the vitamin intake to from 400 to 600 units daily resulted in an increased rate of linear growth. In a large outpatient study, Peatman and Higgons [14a] report the linear growth of 1,112 infants given 1,600 to 9,000 units of vitamin D daily. The rates of growth observed were only average according to recent standards (chart 2). It thus appears that the effect of vitamin D on linear growth during infancy reaches a maximum when the intake is somewhere between 135 and 1,500 units a day. From published reports, the maximal influence is probably in the neighborhood of 300 to 600 units a day.[8]

Present knowledge of the relationship of vitamin D to growth helps to explain the occasional diagnosis of rickets by roentgenographic means when rickets probably is not present. The small changes used as criteria in the roentgenographic diagnosis of slight rickets occur when no vitamin D is given and tend ultimately to increase, producing the picture of clinical rickets. When from 60 to 135 units of vitamin D is given daily, a minimum number of these changes is reported in inpatient studies.[7a] When from 300 to 400 units of vitamin D is given and growth is more rapid, a larger number of small changes in growth at the epiphysial line is reported, but these changes (in inpatient studies) never become any more definitely rachitic and accompany excellent retention of calcium with maintenance of serum calcium and phosphorus at high normal levels. From outpatient studies fewer bone changes are reported when large amounts of vitamin D are given. As the rate of growth seems to be nearly maximal with from 300 to 500 units of vitamin D,[8] it seems that the small changes reported as evidence of early or very mild rickets may represent equally well changes due to rapidity of growth except when no vitamin D is given.

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[14] Jeans, P. C., and Stearns, Genevieve: The Effect of Vitamin D on Linear Growth in Infancy, II. The Effect of Intakes above 1800 U. S. P. Units Daily, J. Pediatrics **13**: 730-740 (Nov.) 1938.

[14a] Peatman, J. G., and Higgons, R. A.: Growth Norms from Birth to the Age of Five Years: A Study of Children Reared with Optimal Pediatric and Home Care, Am. J. Dis. Child. **55**: 1233 (June) 1938.

Factors other than those which have been discussed must be considered along with the vitamin D requirement. One of these is the relative ease of administration. This has been brought out in several published studies of outpatient groups in which it was found that the amount of rickets occurring was in direct proportion to the amount of cod liver oil prescribed.[15] The apparent supremacy of vitamin D milks in outpatient studies may be ascribed to this cause.

Another factor requiring consideration is the relative efficacy of the different varieties of vitamin D. This question has been discussed at length in a previous review.[7a] It is possible that vitamin D from vegetable sources (related to ergosterol) is less potent for man on a unit for unit basis than that from animal sources (related to cholesterol). The difference in potency between these two varieties, if any exists, is small, almost certainly no greater than 1: 1.5, and probably less than this. Criteria capable of determining such a narrow difference must be most exacting, and a satisfactory answer to the problem does not seem possible with available information.

A factor of apparently greater importance than the source of vitamin D is its state of concentration or dispersion. It has long been believed on the basis of clinical experience that a greater unitage of vitamin D is required as viosterol in oil than as cod liver oil. It seems probable that a large part of this difference, if not all of it, may be explained on the basis of relative dispersion. Lewis,[16] Erben[17] and Shelling[18] have shown in clinical experiments that irradiated ergosterol in a dispersed state is much more effective than the same material in a high concentration. The best concentration for efficient utilization is not known. Data[12a] (from observations of infants receiving cod liver oil and various vitamin D milks) indicate that infants can utilize vitamin D in concentrations up to at least 100 units to the gram as efficiently as they utilize this vitamin in a highly dispersed state. The difference in utilization depending on concentration makes difficult the evaluation of data on the effect of higher dosages, for these high dosages have been given almost exclusively as concentrates or patent pharmaceutic preparations. The effect of the very high dosages in decreasing appetite suggests that a larger absolute amount is absorbed than with the smaller dosages of the dispersed forms; the disproportionate antirachitic effect between concentrated and dispersed forms indicates that a much smaller proportion is absorbed from the concentrates. In view of the strong probability that concentrated forms of vitamin D are not fully absorbed, it seems wise to proceed cautiously in administering to infants high unitages of the more dispersible forms of vitamin D. The actual limit of tolerance may be lower than the amounts which hitherto have been administered without toxic effect.

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[15] Drake, T. G. H.; Tisdall, F. F., and Brown, Alan: A Study ot the Relative Antirachitic Values of Cod Liver Oil, Viosterol and Irradiated Milks, Canad. M. A. J. **31**: 368 (Oct.) 1934. Eliot, Nelson, Barnes, Browne and Jenss.[10]

Tentatively the vitamin D requirement of the infant fed cow's milk may be stated as between 300 and 400 units daily, these amounts having been shown to be efficient in prophylaxis of rickets, in producing ample retention of calcium and phosphorus and in permitting excellent skeletal growth and dentition. This requirement is stated in terms of vitamin D of no greater concentration than is found in average high grade cod liver oil.

Infants Fed Human Milk.—The requirement of breast-fed infants for vitamin D is in general less than that of babies fed cow's milk. Infants fed human milk are less liable to the development of rickets when no vitamin D is given; however, rickets is by no means rare. A study of the literature shows that the calcium retention of infants receiving human milk and no additional vitamin D is somewhat less variable than that of infants receiving cow's milk without vitamin D; but the number of low retention values observed is sufficient to account for the occasional development of rickets. In our laboratory four infants fed human milk were given from 300 to 400 units of vitamin D daily; the calcium retention was less variable than is reported for infants given no additional vitamin D, and the average retention was approximately 50 per cent higher. It seems that for infants fed human milk, as for artificially fed babies, the ingestion of vitamin D decreases the range of retention by increasing the efficiency of those with poor retentions to equal that of infants most efficient without vitamin D. It appears that for many breast-fed babies vitamin D is essential and for most of them it is useful. Though the requirement cannot be stated with accuracy on the basis of available data, it would seem wise to prescribe the same amount as is required by the artificially fed baby.

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[16] Lewis, J. M.: Clinical Experience with Crystalline Vitamin D: The Influence of the Menstruum on the Effectiveness of the Antirachitic Factor, J. Pediat. **6:** 362 (March) 1935; Further Observations on the Comparative Antirachitic Value of Crystalline Vitamin D Administered in Milk, Corn Oil, or in Propylene Glycol, ibid. **8:** 308 (March) 1936.

[17] Erben, Fridtjof: Ueber den Einfluss des Lösungsmittels auf die therapeutische Wirksamkeit des bestrahlten Ergosterins, München. med. Wchnschr. **82:** 1794 (Nov. 8) 1935.

[18] Shelling, D. H.: Calcium and Phosphorus Studies: XIII. The Effect of Emulsification on the Potency of Viosterol in Treatment of Rickets in Children, J. Pediat. **10:** 748 (June) 1937.

Prematurely Born Infants.—The vitamin D needs of prematurely born infants are not easy to determine because of the difficulty of supplying ample amounts of calcium and phosphorus during the first few weeks of extra-uterine life. Normally during the last month of full term pregnancy the fetal need of calcium is approximately 0.3 Gm. daily. Obviously it is not possible for a prematurely born infant fed human milk to ingest this quantity of calcium, much less to retain it. Even when the feedings are fortified with dried skimmed milk or calcium caseinate, the total calcium intake is usually under 0.6 Gm., an amount which may be considered scarcely sufficient to permit retention of 0.3 Gm. The prematurely born infant tends to grow more rapidly than the infant born at term. The combination of increased growth and deficient intake is such a handicap that it is doubtful whether the ingestion of vitamin D can compensate entirely, though it may readily compensate to the extent of preventing rickets.

Davidson, Merritt and Chipman[10d] reported that irradiated evaporated milk is inadequate to protect prematurely born infants from moderate or severe rickets. The amount of vitamin D received by these babies varied from 24 to 59 units daily at the age of 1 month. These same authors[10e] found that cow's milk containing 430 units of vitamin D to the quart protected against moderate and severe rickets but not against the roentgen signs of slight rickets. Reinterpretation of the data in the light of the preceding discussion of roentgenographic evidence of rickets in this review permits the exclusion of these cases of slight rickets and allows the conclusion that all the infants were protected. The amount of vitamin D received by these babies varied from 116 to 245 units with an average of 170 units at the age of 1 month. The average birth weight of the babies was 2,000 Gm., and the average weight at 1 month was 2,500 Gm. Horesh and Russell[19] found complete protection against rickets in prematurely born infants from the use of a protein milk containing 3.8 cc. of cod liver oil to the quart. McQuarrie[12b] obtained rickets prevention with 540 units daily as activated ergosterol. Davidson, Merritt and Chipman[20] reported that viosterol fed at a level of 3,150 units daily did not protect against roentgenographic evidence of slight rickets in prematurely born infants receiving human milk, cow's milk or mixed feeding, but again reinterpretation permits the conclusion that rickets was prevented. Shelling[21] observed osteoporosis but not rickets in premature infants given from 3,500 to 4,500 units of vitamin D as viosterol. These reports indicate that rickets is prevented in prematurely born infants by an amount of vitamin D approximately twice that known to protect the majority of full term infants and that rickets is not prevented by an amount less than that required for full term infants. No means are at hand for comparing the relative effects of the same amount of vitamin D in the two groups. The possibility remains that the prematurely born baby requires no more vitamin D for the prevention of rickets than the baby born at term. For the full term infant the amount that permits ample retention of calcium is considerably greater than that which barely prevents rickets. No doubt this holds for the prematurely born baby also. Until further information is obtained it may be tentatively considered that prematurely born babies require twice as much vitamin D as babies born at term, or from 600 to 800 units fed in a dispersed form. After the period of most rapid growth in early infancy the requirement should be the same as for the full term infant.

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[19] Horesh, A. J., and Russell, G. R.: Observations on the Growth and State of Nutrition of Premature Infants Given an Antirachitic and Antiscorbutic Food, Ohio State M. J. **31:** 339 (May) 1935.

[20] Davidson, L. T.; Merritt, Katharine K., and Chipman, S. S.: Further Studies of Viosterol in the Prophylaxis of Rickets in Premature Infants, Am. J. Dis. Child. **51:** 594 (March) 1936.

[21] Shelling, D. H., and Hopper, Katherine B.: Calcium and Phosphorus Studies: XII. Six Years' Clinical Experience with Viosterol in Prevention and Treatment of Rickets, Tetany and Allied Diseases, Bull. Johns Hopkins Hosp. **58:** 137 (March) 1936.

REQUIREMENT DURING CHILDHOOD

The requirement for ingested vitamin D has been assumed to decline after infancy, though little experimental evidence for this supposition exists. Belief in this idea is reflected in the widespread lack of use of vitamin D preparations in childhood compared to the almost universal use in infancy. The assumption of a lesser need may be based on the absence of rickets in childhood and on the presumption that older children get more sunshine than infants. Whether or not the fault is with vitamin D, there is abundant evidence that present customs of diet in this country are not fully adequate for optimal skeletal and dental development. This is attested by the prevalence of dental caries and, in late childhood, the finding of osteoporosis. As a cause of these abnormalities a deficiency of intake of calcium, vitamin D or both substances is probably more common with the American type of diet than an intake deficient in phosphorus. At least some of these defects may be caused by inadequacy of vitamin D.

The criteria of adequacy of intake of vitamin D are fewer for children than for infants. Prevention of rickets is of no value and studies of levels of calcium and phosphorus in the blood are of little assistance. The rate of growth might be useful, but no studies have come to our attention in which this criterion was used when vitamin D was the only factor varied. A criterion which has a considerable degree of usefulness is the prevention and arrest of dental caries. The most satisfactory criterion subject to quantitative measurement is the retention of calcium and phosphorus with and without vitamin D, compared with the calculated requirement for these minerals.

Dental Caries.—Numerous observations have shown that vitamin D is a factor in prevention and arrest of dental caries. In some instances vitamin D has been added to the diet in an orphanage or other group without other change. The effect on the teeth has been variable, as is to be expected. It is worth reiterating that vitamin D does not reduce the minimum requirement for calcium and that vitamin D may not be expected to improve a diet containing an amount of calcium below the minimum requirement. In order to determine the vitamin D requirement by using the criterion of dental caries, the diet must be adequate in all respects other than vitamin D, including adequate calcium and phosphorus.

Mellanby [22] administered cod liver oil in an amount containing from 700 to 1,500 units of vitamin D daily as a supplement in orphanage diets which happened to be deficient in fruits and animal protein and which contained about 500 cc. of milk daily. Mellanby reported that the children to whom cod liver oil had been given developed fewer new cavities and showed arrested progress of old caries, although carious activity was not entirely prevented. A somewhat similar type of observation by Day and Sedwick [23] gave negative results. They found no change in the carious processes in a group of school children for whom was prescribed 4,000 units of vitamin D daily as viosterol, as compared to a control group not receiving vitamin D. These children lived at home and the diets were uncontrolled. Anderson and his co-workers [24] noted a "marked decrease" in dental caries when viosterol (4,700 units) was administered to children with presumably good diets. McBeath [25] reported reduced incidence of dental caries in an orphanage group whose diets are recorded as "good," when 10,000 units of vitamin D as viosterol was given daily without other dietary change. It is evident from the observations cited and others in the literature that vitamin D is a factor in the arrest and control of dental caries, but most of these reports give no clue to the vitamin D requirement.

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[22] The Committee for Investigation of Dental Disease: The Influence of Diet on Caries in Children's Teeth (Final Report), Medical Research Council, Special Report Series, No. 211, London, His Majesty's Stationery Office, 1936.

[23] Day, C. D. M., and Sedwick, H. J.: The Fat Soluble Vitamins and Dental Caries in Children, J. Nutrition **8**: 309 (Sept.) 1934.

[24] Anderson, P. G.; Williams, C. H. M.; Halderson, H.; Summerfeldt, C., and Agnew, R. G.: The Influence of Vitamin D in the Prevention of Dental Caries, J. Am. Dent. A. **21**: 1349 (Aug.) 1934.

[25] McBeath, E. C.: Nutritional Control of Dental Caries, New York State J. Med. **33**: 1086 (Sept. 15) 1933.

McBeath[26] has reported the prevention of dental caries with the administration of an excellent experimental diet including 3 teaspoonfuls of cod liver oil. He[26] has reported also in a winter study of orphanage children a decrease in the number of new carious processes when the unrecorded diet was altered by the addition of 1 pint of milk and by 1 pint of milk containing 270, 400 and 800 units of vitamin D respectively, the improvement in the dental condition being in the order stated. Decrease in caries produced by the addition of milk alone indicates that the basal diet may not have been adequate. The apparent superiority of 800 over 400 units of vitamin D may not be actual, because these unitages were employed in different orphanages and were not added to the same basal orphanage diet. However, definite superiority of 800 over 270 units was shown.

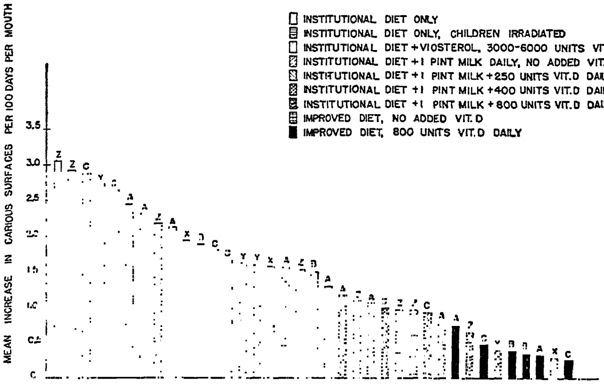

Chart 3.—The effect of improved diet and vitamin D on reducing the incidence of dental caries. Data from winter studies of McBeath and Zucker.[26b] The letters indicate the orphanages studied. The control studies (hollow columns) show the variation according to orphanage and year of study. While the experimental data are not strictly comparable according to orphanage and year, it seems clear that when the intake of calcium is sufficient, the ingestion of ample amounts of vitamin D causes a consistent reduction in the incidence of dental caries, and that the ingestion of a good diet and ample vitamin D reduces the incidence markedly.

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[26] (*a*) McBeath, E. C.: Vitamin D Studies, 1933-1934, Am. J. Pub. Health **24:** 1028 (Oct.) 1934. (*b*) McBeath, E. C., and Zucker, T. F.: The Role of Vitamin D in the Control of Dental Caries in Children, J. Nutrition **15:** 547 (June) 1938.

Boyd and Drain and their co-workers[27] observed rapid arrest of dental caries in children given a well rounded diet which included a quart of milk and about 350 units of vitamin D daily, the latter in the form of cod liver oil. In a recent intensive study of four children with dental decay these authors[28] noted the effect of diets otherwise adequate but with the vitamin D intakes graduated; 0, 155 and 600 units daily were given successively to the same children. The ingestion of a good diet without added vitamin D brought about appreciable lessening of the activity of the caries during the first few weeks of the study in the fall months with the children spending much time outdoors. Subsequently for a period of five months, during which the children received a diet of high protective value aside from its vitamin D content and through both the periods of no added vitamin D and of the 155 unit addition, the caries was stationary with minimal but definite activity. It was not until the higher amount of 600 units of vitamin D had been given for nine weeks that the caries became definitely arrested. The results with 600 units daily showed no advantage of this amount over the 350 units daily intake of previous experiments.

Incidentally it is worthy of mention that the studies of Boyd and Drain have pertained exclusively to dentinal caries, while most if not all other observers have included both caries of the dentin and of the enamel in their data. Included in the latter designation, lesions due to dystrophy, fracture and disintegration have been considered; any surface irregularities deep enough to catch the exploring tine have been designated as sites of decay. Obviously not all these bear relation to nutrition. This difference of interpretation must be evaluated in comparing studies of tooth decay. The effect of nutrition on enamel disintegration remains to be determined. It is possible that complete prevention of enamel "caries" by a nutritional regimen is not to be expected.

One may conclude from the dental studies that the greatest freedom from dental caries is observed in groups of children receiving an adequate diet containing ample quantities of milk and 350 or more units of vitamin D daily. If the diet is suboptimal, the addition of vitamin D lessens the severity but does not prevent dental caries. Similarly, a diet otherwise adequate but lacking in vitamin D may decrease the incidence but not prevent entirely the development of tooth decay, nor provide for complete arrest of caries already present.

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[27] Boyd, J. D.; Drain, C. L., and Nelson, Martha V.: Dietary Control of Dental Caries, Am. J. Dis. Child. **38**: 721 (Oct.) 1929.

[28] Boyd, J. D.; Drain, C. L., and Stearns, Genevieve: Nature of Diet in Its Relationship to Control of Dental Caries, Proc. Soc. Exper. Biol. & Med. **36**: 645 (June) 1937.

Calcium and Phosphorus Retention.—Many studies of calcium and phosphorus retention of children are available, though in but few have comparative studies been made with and without vitamin D. Unlike studies throughout infancy, wherein the dietary regimen is known for the entire period after birth, observations in childhood are more likely to be handicapped by incomplete knowledge of the previous dietary regimen. Wang [29] has shown that if the child's previous diet has been deficient in calcium, the calcium retention during the period of observation may be unusually high. In our studies there have been observed also a few children whose previous diets had been deficient in calcium but who reacted very slowly to the change in diet; only after several weeks of ingestion of an adequate diet with added vitamin D did the calcium and phosphorus retention reach the average of the group. Because of the influence of previous dietary conditions on the retention observed from a given diet, it has become customary to allow a longer period of adjustment to the experimental diet than was formerly the custom and to make a series of retention studies rather than a single observation with each given dietary. When the experiment is conducted in this manner the results are more reliable.

The adequacy of a diet in calcium, phosphorus and vitamin D may be judged by comparing the retention observed with the quantities considered ample for osseous and dental development. Because the quantity of phosphorus retained varies with the nitrogen as well as the calcium retention, the quantity of calcium retention may be used as the most convenient guide. When the standards set up by Leitch[5c] are recalculated into the more customary terms of retention per kilogram, the calcium retention considered desirable decreases from

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[29] Wang, Chi Che; Kaucher, Mildred, and Frank, Margaret: Metabolism of Undernourished Children: IV. Calcium Metabolism, Am. J. Dis. Child. **35**: 856 (May) 1928.

25 mg. per kilogram for a year old child to from 10 to 12 mg. for a child over 6 years of age. These values are somewhat higher for young children than those postulated as desirable by Sherman and Hawley.[5b]

Reports of long term studies of children not given vitamin D stress the variability of calcium retention observed in a group of children [30] or in the same child from period to period of study.[31] The retention observed with an intake of 1 Gm. of calcium daily varied from 6 to 24 mg. per kilogram, and the average retention of some children was far below the amounts postulated as safe by Leitch.

Weld and Sykes [32] determined the calcium and phosphorus retention of six children over a five week period with an intake of 0.82 Gm. of calcium and no added vitamin D. During a second five week period 135 units of vitamin D as irradiated ergosterol was added to the diet of four of the children with no significant change observed in the retention.

We [33] studied fifty children aged from 1 to 12 years with varying intakes of milk and with and without added vitamin D. When given, the amount of vitamin D was from 300 to 400 units as cod liver oil daily. For the majority of these children each dietary regimen was maintained for from five to eight weeks. A few were studied for periods up to three months with one dietary regimen. All children were permitted outdoor exercise when weather allowed. The variability of retention noted by others when children were not given vitamin D was also observed in this series. The retention of the younger children was more variable than that of the older but less variable than had been observed in infants not given vitamin D. As with the infants, the addition of vitamin D decreased the variability of retention by decreasing the number of poor retentions. Thus, if a child utilized calcium efficiently without added vitamin D, the addition of the vitamin to the diet had little effect. If, however, the utilization was poor, the ingestion of vitamin D increased the retention of calcium. The increased retention was by no means always observed promptly; two of the children studied did not achieve retention equal to the average of their age group until the diet with vitamin D had been given for a period of from two to three months. One of these children had active dental caries at the beginning of the experiment; the caries remained active much longer than is customarily observed in this clinic. The retention observed in this group of fifty children indicates that the ingestion of vitamin D in no way decreases the quantity of calcium need but that it assures better utilization. A retention equal to that postulated by Leitch as desirable was consistently obtained when the milk intake was 750 cc. daily or higher and when vitamin D was given.

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[30] Hunscher, Helen A.; Hummel, Frances C., and Macy, Icie G.: Variability of Metabolic Response of Different Children to a Given Intake of Calcium, Proc. Soc. Exper. Biol. & Med. **35:** 189 (Oct.) 1936.

[31] Porter-Levin, Thelma: Calcium and Phosphorus Metabolism of Normal Preschool Children: II. Successive Balance Studies Showing Range of Variation in Calcium and Phosphorus Studies, J. Am. Dietet. A. **9:** 22 (May) 1933.

[32] Weld, C. B., and Sykes, J. F.: The Effect of Irradiated Ergosterol on Calcium and Phosphorus Retention in Children, Tr. Roy. Soc. Canada (Sect. V) **26:** 81, 1932.

[33] Jeans, P. C., and Stearns, Genevieve: The Vitamin D Requirement of the Child, Proc. Am. Pediat. Soc., Am. J. Dis. Child. **54:** 189 (July) 1937.

Daniels and her collaborators[34] fed vitamin D in daily amounts varying from approximately 1,200 units (15 cc. of cod liver oil) to 3,000 units or more (15 cc. of cod liver oil plus 8 drops of viosterol). Though these studies were of short duration, the study group was large. An average retention of about 10 mg. per kilogram was observed whether the intake of milk was 1 pint or 1 quart daily. These retention values are lower than those noted by Hunscher and her co-workers in long term studies of children of this age group not receiving additional vitamin D, and considerably lower than the average values observed by us in children of the same age range given from 300 to 400 units of vitamin D daily.

It seems from a summary of the studies which have been cited that the variability of response to the calcium of the diet is marked in young children not given vitamin D. Efficiency of utilization tends to increase with increasing age of the child, but even by 9 or 10 years of age many children seem unable to utilize efficiently the calcium and phosphorus of the diet unless vitamin D is also ingested. The daily allowance of at least 750 cc. of milk together with from 300 to 400 units of vitamin D seems to permit consistently ample retention of calcium and phosphorus for proper development of bone and teeth. The data at hand are not sufficient to allow an accurate estimate of the optimal intake of vitamin D for children, though it appears probable that the total quantity needed daily is not greater than the amount required for infants, nor does it seem to be less.

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[34] Daniels, Amy L.; Hutton, Mary K.; Knott, Elizabeth; Everson, Gladys, and Wright, Olive E.: Relation of Ingestion of Milk to Calcium Metabolism in Children, Am. J. Dis. Child. **47**: 499 (March) 1934. Daniels, Amy L.; Hutton, Mary K.; Knott, Elizabeth; Wright, Olive, and Forman, Mary: Calcium and Phosphorus Needs of Pre-School Children, J. Nutrition **10**: 373 (Oct.) 1935.

ADOLESCENCE

The literature contains very few reports of studies of either the calcium or the vitamin D requirement of adolescents, notwithstanding the fact that this age period is of special importance because of the rapid skeletal growth. Leitch has estimated that a retention of from 500 to 700 mg. of calcium daily is necessary to maintain normal calcification during this period. The condition of the teeth of the average adolescent and the possible occurrence of osteoporosis [6a] during this period of growth indicate inadequacy of present dietaries. Whether in this state of suboptimal calcification the need is primarily for calcium or for vitamin D is difficult to determine with the data at hand.

Herbst [35] in 1913 reported calcium retention of two boys aged 13 and 14 years, with a calcium intake of 0.62 and 0.64 Gm. daily. The younger, who was growing rapidly, retained 0.3 Gm. of calcium daily, the older 0.15 Gm. Wang and her associates [36] have summarized the scanty data in the older literature and have added twenty-three six day studies of girls from 12 to 15 years of age. Vitamin D was not given. In Wang's study the daily intake of calcium varied from 1.18 Gm. to 1.80 Gm. and the retention from 0.079 Gm. to 0.823 Gm., or from 1 to 19 mg. with an average of 11 mg. per kilogram; the average retention was 417 mg. daily, two thirds of the group retaining less than the Leitch standard of 500 mg. daily.

Henderson and Kelly [37] studied for twenty-four weeks the calcium retention of five African boys aged from 15 to 17 years. With the control diet containing

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[35] Herbst, O.: Calcium und Phosphor beim Wachstum am Ende der Kindheit, Ztschr. f. Kinderh. **7:** 161, 1913.

[36] Wang, Chi Che; Kaucher, Mildred, and Wing, Mary: Metabolism of Adolescent Girls: IV. Mineral Metabolism, Am. J. Dis. Child. **52:** 41 (July) 1936.

[37] Henderson, J. M. and Kelly, F. C.: Influence of Certain Dietary Supplements in Relation to the Calcium Requirements of Growing African Natives, J. Hyg. **29:** 429 (Feb.) 1930.

0.3 Gm. of calcium the boys lost calcium at a rate of approximately 0.1 Gm. a day. The addition of cod liver oil to the diet of one boy did not decrease the calcium loss. The diets of three of the subjects were then supplemented respectively with 1 pint of milk daily, a mineral mixture equivalent to the minerals of 1 pint of milk daily, and the mineral mixture with added cod liver oil. The calcium retention of each of the three boys was consistently positive, varying from 0.096 Gm. to 0.5 Gm. daily, the higher value being obtained with the addition of milk without cod liver oil. The data are insufficient to permit evaluation of the effect of vitamin D added to the increased diet but do demonstrate that the ingestion of vitamin D did not decrease the minimal requirement for calcium.

The data obtainable from studies of adolescents indicate that individuals are by no means equally efficient in retaining calcium without the ingestion of vitamin D. Apparently the variability in ability to utilize the calcium and phosphorus of the diet is a major factor in determining the quantity of these elements retained by adolescents as well as by younger children. It is indicated also that vitamin D does not lower the minimum requirement for ingested calcium, though no estimate can be made as to the optimal intake of either calcium or vitamin D.

REQUIREMENTS OF ADULTS

Men and Nonpregnant Women.—During adult life the quantity of calcium and phosphorus considered necessary is only that amount sufficient for maintenance. It may be found that some retention of calcium and phosphorus is desirable, at least during the post-adolescent period. Whether the addition of vitamin D may be necessary to insure such retention or whether the ingestion of vitamin D would insure better utilization of an intake only slightly above minimal requirements are questions difficult to answer with the few data at hand.

Much of the calcium intake of adults may be from sources other than milk; therefore the acid-base ratio of the diet assumes importance. The ingestion of an acid-ash diet or of acid salts increases the urinary excretion of calcium. If the intake is low, the added excretion may be sufficient to cause a negative calcium balance. Wide differences in intake ratios of calcium and phosphorus apparently have less effect on the retention of these elements by adults[38] than by children.[39]

Leitch[5c] has summarized the data for the calcium requirement of women not ingesting vitamin D. She concluded that, at or above a daily intake of 0.55 Gm. of calcium, losses of one period were equaled by gains of another, whereas below this intake output usually exceeded intake. The minimum calcium requirement as set by Sherman[40] is somewhat lower; namely, 0.45 Gm. a day. Few data are available concerning the requirement for men, but such as there are[5c] indicate that the daily requirement is about the same as for women.

Most of the published studies of the effect of vitamin D on calcium and phosphorus retention of adults have centered on an attempt to determine whether an increased ingestion of vitamin D decreases the minimal requirement for calcium. The answer is unanimously in the negative.[41] When vitamin D has been given as an addition to a diet containing more than the minimal requirement of calcium, the results have been conflicting. All the subjects studied by Kelly and Henderson[41] and by Bauer and his co-workers[41] retained calcium when the intake of this element was ample and no vitamin D was ingested. A further increase in retention was noted by Kelly and Henderson when cod liver oil was given in addition to the increased mineral intake. Bauer and his collaborators observed no increase in retention when from 30 to 90 mg. of irradiated ergosterol was given in addition to the high calcium diet but did obtain evidence of increased absorption, for the urinary excretion of calcium was increased and the fecal calcium correspondingly decreased when vitamin D was given. Hunscher and her co-workers[42] studied three young women whose calcium intake varied from 0.7 to 1.0 Gm. a day, amounts ordinarily considered ample. When no vitamin D was given two of the three women consistently lost 0.1 Gm. of calcium a day, the third was approximately in calcium equilibrium. When 15 Gm. of cod liver oil was given daily for twenty-three days the average calcium losses were not decreased, nor do the data show any significant alteration in the mode of calcium excretion.

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[38] Farquharson, R. F.; Salter, W. T., and Aub, J. C.: The Effect of the Ingestion of Phosphates on the Excretion of Calcium, J. Clin. Investigation **10:** 251 (June) 1931.

[39] Stearns, Genevieve, and Jeans, P. C.: Utilization of Calcium Salts by Children, Proc. Exper. Biol. & Med. **32:** 428 (Dec.) 1934.

[40] Sherman, H. C.: Calcium Requirement of Maintenance in Man, J. Biol. Chem. **44:** 21 (Oct.) 1920.

[41] Hart, M. C.; Tourtellotte, Dee, and Heyl, F. W.: Effect of Irradiation and Cod Liver Oil on Calcium Balance in the Adult Human, J. Biol. Chem. **76:** 143 (Jan.) 1928. Kelly, F. C., and Henderson, J. M.: The Influence of Certain Dietary Supplements on the Nutrition of the African Native: I. J. Hyg. **29:** 418 (Feb.) 1930. Bauer, Walter; Marble, Alexander, and Claflin, Dorothy: Studies on the Mode of Action of Irradiated Ergosterol: I. Its Effect on the Calcium, Phosphorus and Nitrogen Metabolism of Normal Individuals, J. Clin. Investigation **11:** 1 (Jan.) 1932.

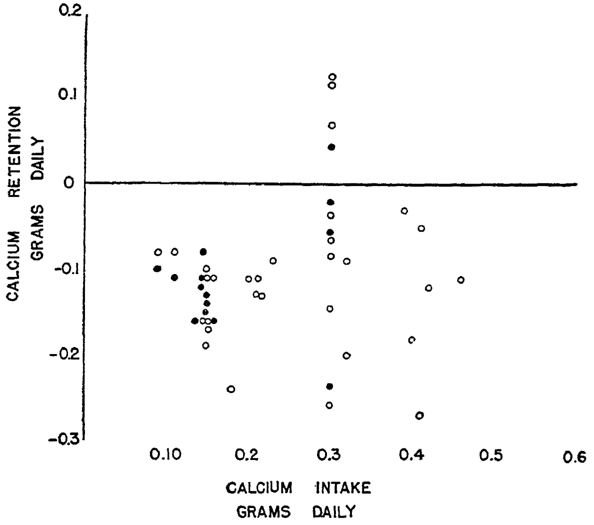

Chart 4.—The ineffectiveness of vitamin D when the calcium intake is subminimal. Data from the literature[40, 41] showing calcium balance studies of normal adults ingesting diets low in calcium. The solid symbols indicate studies wherein vitamin D was ingested in amounts equivalent to or greater than 12 cc. of cod liver oil; the hollow symbols indicate periods when no vitamin D was given. These studies demonstrate that vitamin D is not a substitute for calcium.

Data are not available as to the effect of vitamin D on retention of calcium and phosphorus by middle-aged or elderly persons. The prevalence of senile osteoporosis may be due wholly to the habit common to most American adults of ingesting diets low in calcium. It has been shown[43] that mineralization of bone can be increased in adults past 60 by increasing the intake of calcium and vitamin D. Since elderly persons are less likely to be exposed to sunlight than more vigorous younger adults, the ingestion of moderate amounts of vitamin D, together with a diet ample in calcium and phosphorus, might serve to prevent the development of osteoporosis in the older adult.

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[42] Hunscher, Helen A.; Donelson, Eva, and Erickson, Betty Nims: Results of the Ingestion of Cod Liver Oil and Yeast on Calcium and Phosphorus Metabolism, J. Nutrition **8**: 341 (Sept.) 1934.

[43] Meulengracht, E., and Meyer, A. R.: Ugeskr. f. laeger. **98**: 961, 1936; quoted by Leitch.[5c]

Animal studies bearing on these questions include those of Sherman and Campbell,[6b] who showed that when young rats are given a diet somewhat subminimal in calcium content, normal growth occurs, but normal calcification of bone is not achieved until nearly middle age. A recent report of these authors [44] lists an ample intake of calcium among the factors resulting in longer life and extension of the period of adult vitality of rats. Even if these observations are assumed to hold for human beings, there still remains to be determined whether and how much vitamin D would contribute to better utilization of calcium by adults.

The observations on adults indicate, as for children, that the ingestion of vitamin D in no way lessens the requirement for calcium. Whether vitamin D, when given in addition to an ample mineral intake, increases the utilization of calcium undoubtedly varies with the person. Although the average adult seems more efficient in the absorption of calcium than the average child, vitamin D may aid the inefficient adult in the same manner as the inefficient child. If this is true, the amount of vitamin D optimal for this purpose remains to be determined.

Reed and others [45] have given vitamin D in enormous dosage to adults, with apparently few untoward effects. The limit of tolerance varies with the individual but seems to be 150,000 units or more. The vitamin in these studies was given always as a very concentrated product. Whether adults utilize the concentrated sources of vitamin D efficiently or not, the enormous unitage given can be interpreted as indicating that the tolerance of the adult to vitamin D must be relatively high.

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[44] Sherman, H. C., and Campbell, H. L.: Nutritional Well-Being and Length of Life as Influenced by Different Enrichments of an Already Adequate Diet, J. Nutrition **14:** 609 (Dec. 10) 1937.

[45] Reed, C. I.: Symptoms of Viosterol Overdosage in Human Subjects, J. A. M. A. **102:** 1745 (May 26) 1934. Wyatt, B. L.; Hicks, R. A., and Thompson, H. E.: Massive Doses of Vitamin D in the Treatment of Proliferative Arthritis, Ann. Int. Med. **10:** 534 (Oct.) 1936. Steck, I. E.: Clinical Experience in the Treatment of Arthritis with Massive Doses of Vitamin D, Illinois M. J. **71:** 243 (March) 1937. Ceder, E. T., and Zon, Leo: Treatment of Psoriasis with Massive Doses of Crystalline Vitamin D and Irradiated Ergosterol, Pub. Health Rep. **52:** 1580 (Nov. 5) 1937.

Pregnancy and Lactation.—The calcium and phosphorus requirement during pregnancy and lactation has received much intensive study. The report of Bauer and Aub [46] indicates that with a subminimal intake the pregnant woman is unable to conserve calcium for the needs of the fetus. The Toveruds [47] observed that when the mother's diet was deficient, the skull of the infant was softer than normal, the epiphysial lines, particularly of the metacarpals, were fringed and poorly calcified, and the teeth when erupted were often grossly hypoplastic. They observed further that when the mother's diet contained ample calcium and vitamin D, the bones and teeth of the infant were normal. Finola [48] and his co-workers noted a significant increase in the density of bone and a decrease in the size of the fontanel of babies whose mothers received a good diet plus approximately 1.5 Gm. of calcium as dicalcium phosphate and 7,000 units of vitamin D as viosterol, in comparison with infants whose mothers received a good diet without the addition of calcium and vitamin D.

The Toveruds [47] and Mellanby [49] both consider a poor diet of the mother during pregnancy as one of the chief predisposing factors in the development of rickets and dental caries in the children. Calcium and vitamin D are two of the factors most often deficient in the mother's diet. Corroborative evidence of the need of increased mineral retention in pregnancy is obtained in the study of Drain and Oberst [50] wherein the dental condition of pregnant women was shown to be improved by a diet rich in calcium and vitamin D.

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[46] Bauer, Walter; Albright, Fuller, and Aub, J. C.: Studies of Calcium and Phosphorus Metabolism: II. The Calcium Excretion of Normal Individuals on a Low Calcium Diet; also Data on a Case of Pregnancy, J. Clin. Investigation **7:** 75 (April) 1929.

[47] Toverud, K. U., and Toverud, G.: Studies on Mineral Metabolism During Pregnancy and Its Bearing on Disposition to Rickets and Dental Caries, Acta paediat. **12:** (supp. 2) 1-116, 1931.

[48] Finola, G. C.; Trump, Ruth A., and Grimson, Mozelle: Bone Changes in the Fetus Following the Administration of Dicalcium Phosphate and Viosterol to the Pregnant Mother, Am. J. Obst. & Gynec. **34:** 955 (Dec.) 1937.

[49] Mellanby, May: Diet and the Teeth: III. The Effect of Diet on Dental Structure and Disease in Man, Medical Research Council, Special Report Series, no. 191, London, His Majesty's Stationery Office, 1934.

[50] Drain, C. L.; Plass, E. D., and Oberst, W. F.: Prevention and Control of Dental Caries in Pregnant Mothers, J. Dent. Research **13:** 233 (April) 1933.

The work of many investigators [51] demonstrates that a calcium intake of at least 1.4 to 1.6 Gm. daily is necessary in order to permit retention of the amount of calcium needed by the fetus. Below this intake the addition of vitamin D does not insure retention. With a calcium intake of from 1.6 to 2.5 Gm. some persons can retain calcium in ample amounts even without added vitamin D. Studies of multiparas [52] show that the drain of rapidly succeeding pregnancies and periods of lactation may result in poor retention of calcium and phosphorus regardless of intake unless vitamin D is also given. Very few show poor retention with a high intake of calcium even when the vitamin D intake is equivalent to 20 Gm. of cod liver oil. In general the addition of vitamin D in amounts supplied by from 10 to 20 Gm. of cod liver oil daily tends to increase the retention of calcium and phosphorus. Insufficient studies have been made with ample intake of calcium and varying amounts of vitamin D to permit a conclusion or general statement as to the vitamin D requirement during pregnancy.

The studies of lactation [53] indicate that the drain on the mother is more severe than in pregnancy. The need for ample calcium intake is imperative. There seems necessary an intake of at least 1½ quarts of milk, containing 1.8 Gm. of calcium and representing a total day's intake of more than 2 Gm. Even then, relatively few women are efficient enough to prevent loss of calcium from the body unless additional vitamin D is given. Desirable amounts of vitamin D seem to vary with the individual, as shown by the following unpublished study from our laboratory: Two primiparas, aged 21 and 26, ingested good diets, each containing 1,500 cc. of milk and one teaspoonful of cod liver oil (from 350 to 400 units) daily. The 21 year old woman secreted from 1,000 to 1,500 cc. of milk

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[51] (*a*) Garry, R. C., and Stiven, D.: A Review of Recent Work on Dietary Requirements in Pregnancy and Lactation, with an Attempt to Assess Human Requirements, Nutrition Abstr. & Rev. **5:** 855 (April) 1936. (*b*) Macy, Icie G., and Hunscher, Helen A.: Evaluation of Maternal Nitrogen and Mineral Needs During Embryonic and Infant Development, Am. J. Obst. & Gynec. **27:** 878 (June) 1934. (*c*) Hummel, Frances C.; Hunscher, Helen A.; Bates, Mary F.; Bonner, Priscilla; Macy, Icie G., and Johnston, J. A.: A Consideration of the Nutritive State in the Metabolism of Women During Pregnancy, J. Nutrition **13:** 263 (March) 1937. (*d*) Coons, Callie Mae; Schiefelbusch, A. T.; Marshall, G. B., and Coons, R. R.: Studies in Metabolism During Pregnancy, Oklahoma Agriculture and Mechanical College, Agriculture Experiment Station, Bulletin 223, March, 1935. Coons, Callie Mae, and Coons, R. R.: Some Effects of Cod Liver Oil and Wheat Germ on the Retention of Iron, Nitrogen, Phosphorus, Calcium and Magnesium During Human Pregnancy, J. Nutrition **10:** 289 (Sept.) 1935. (*e*) Toverud and Toverud.[47]

[52] Macy, Icie G.; Hunscher, Helen A.; Nims, Betty, and McCosh, Sylvia S.: Metabolism of Women During the Reproductive Cycle: I. Calcium and Phosphorus Utilization in Pregnancy, J. Biol. Chem. **86:** 17 (March) 1930. Hummel, Frances C.; Sternberger, Helen R.; Hunscher, Helen A., and Macy, Icie G.: Metabolism of Women During the Reproductive Cycle: VII. Utilization of Inorganic Elements (A Continuous Case Study of a Multipara), J. Nutrition **11:** 235 (March) 1936.

[53] Macy, Icie G.; Hunscher, Helen A.; McCosh, Sylvia S., and Nims, Betty: Metabolism of Women During the Reproductive Cycle: III. Calcium, Phosphorus and Nitrogen Utilization in Lactation Before and After Supplementing the Usual Home Diets with Cod Liver Oil and Yeast, J. Biol. Chem. **86:** 59 (March) 1930. Hunscher, Helen A.: Metabolism of Women During the Reproductive Cycle: II. Calcium and Phosphorus Utilization in Two Successive Lactation Periods, ibid. **86:** 37 (March) 1930. Toverud and Toverud.[47] Footnote 52.

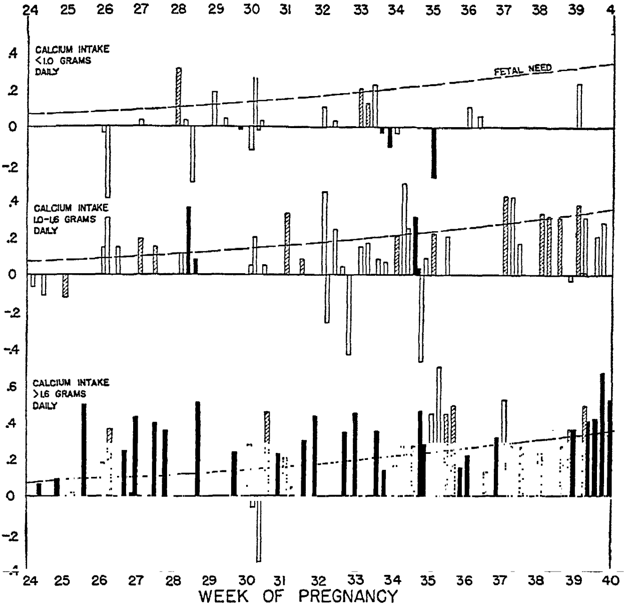

Chart 5.—Calcium retention of pregnant women in relation to intake of calcium and of vitamin D. Data from the literature[47, 51d, 52] and from authors' laboratory. The hollow columns represent periods of study wherein no vitamin D was given; diagonal markings show studies wherein women were exposed to actinic rays of the sun (Oklahoma sunshine[51d] or Iowa summer sun); solid columns represent periods of study when the subjects ingested 400 or more units of vitamin D daily. When the intake of calcium is subminimal (less than 1 Gm. of calcium daily) the effect of exposure to the sun or ingestion of vitamin D is not constant. As the intake of calcium increases, the effect of vitamin D becomes more apparent; with an intake of 1 to 1.6 Gm. of calcium daily, enough calcium is retained for fetal needs; when the intake of both calcium and vitamin D is ample, calcium is stored against the drain of lactation. When no vitamin D is given, even though the calcium intake is ample, storage of calcium is erratic and heavy losses may occur.

daily and retained calcium continuously. The other woman secreted never more than 600 cc. of milk a day yet continuously lost calcium from the body; increasing the calcium intake decreased the loss but did not prevent it entirely. Again, two other primiparas, aged 17 and 19, were given from 1,800 to 2,000 cc. of milk daily. One retained calcium consistently throughout lactation; the other was unable to retain calcium until from 750 to 800 units of vitamin D daily as cod liver oil was given. The milk output of the latter was approximately 1,500 cc. and that of the former approximately 1,000 cc. a day.

Hunscher's studies[53] tend to show that in rapidly succeeding pregnancies and lactation periods vitamin D is especially necessary and that ample calcium intake does not suffice to prevent loss of calcium from the body unless vitamin D is given.

One factor often overlooked is the effect of the maternal diet on the quality of the milk secreted. Telfer[54] commented on the higher mineral content of milk of country women compared to that of women from cities. The differences were ascribed to diet and to the relative amount of sunlight. The Toveruds[47] found a low calcium content in the milk of fourteen mothers whose diets were poor; when the calcium intake was increased the calcium content of the milk of each woman was increased after one week, the average increase being about 25 per cent. The effect of added vitamin D was not studied, but the possibility of its ingestion increasing the calcium content of the milk should not be ignored. The content of calcium in human milk varies considerably according to reports.

While available data point strongly to the advisability of giving vitamin D during pregnancy and lactation, it is impossible with our present information to arrive at any conclusion regarding optimal dosage. Individual variability has been demonstrated. The amount of vitamin D to be recommended is that which is desirable for the least efficient in utilizing the calcium of the diet. This amount may be greater, especially during lactation, than at any other period of life.

Animal experiments wherein large amounts of vitamin D were given to pregnant rats show that, if the maternal diet is good, the calcium and phosphorus content of the bones of the young rats is increased above that of the control group;[55] large doses of viosterol together with a deficient diet result in decreased content of mineral in the bones of young.[55] Subtoxic doses of vitamin D given to rats receiving a diet deficient in minerals during gestation increase the calcium and phosphorus content of the offspring over that of young born of mothers given the deficient diet without added vitamin D.[56] Hypervitaminosis D has not been reported in women during pregnancy and lactation; consequently the effect is unknown.

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[54] Telfer, S. V.: Mineral Metabolism in Infancy: I. The Mineral Constituents of Human Milk and Cow's Milk, Glasgow M. J. **113:** 246 (May) 1930.

SUMMARY

Individual variation in ability to utilize the calcium and phosphorus of the diet without added vitamin D exists at all age periods. A high proportion of infants have poor retention and only a very few retain an ample amount without vitamin D. As the age increases persons in increased proportion are able to retain adequate amounts of these minerals without vitamin D, but at all age periods some persons are found who are not efficient. In defining a standard for the vitamin D requirement it seems desirable to state an amount which will be satisfactory for those who are less efficient. Vitamin D tends to decrease the range of retention of calcium and phosphorus in study groups by increasing the retention of those least efficient to approximate that of those most efficient. Vitamin D ordinarily does not increase the retention of those who have high retention without it.

Vitamin D does not decrease the minimum requirement of calcium and phosphorus and this vitamin cannot produce a good retention in a person who is ingesting less than the requirement for these minerals. Determination of the need for vitamin D must be based on studies of persons who are receiving an ample intake of calcium and phosphorus.

It appears from available evidence that vitamin D is not as well utilized on a unit for unit basis from the more concentrated preparations as from those preparations in which it is more widely dispersed. The

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[55] Nicholas, H. D., and Kuhn, Evelyn M.: Role of Calcium, Phosphorus and Vitamin D in Pregnancy, J. Clin. Investigation **11:** 1313 (Nov.) 1932.

[56] Sontag, L. W.; Munson, Paul, and Huff, Elton: Effects on Fetus of Hypervitaminosis D and Calcium and Phosphorus Deficiency During Pregnancy, Am. J. Dis. Child. **51:** 302 (Feb.) 1936.

most desirable concentration has not been determined, but apparently the concentration found in cod liver oil is as effective as any lesser concentration studied.

On the basis of the preceding premises and of an evaluation of data in the literature the requirement for vitamin D for different age periods and conditions has been tentatively postulated. These requirements are considered in terms of a concentration of vitamin D no greater than is found in average high grade cod liver oil.

The vitamin D requirement of the full term artificially fed baby is probably between 300 and 400 units a day.

Normal babies receiving human milk require less vitamin D than do babies receiving cow's milk, but how much less is not known. However, vitamin D is necessary for many and useful for most breast-fed babies. It would seem wise to prescribe for them the same amount as is required by artificially fed babies.

It is tentatively considered that prematurely born babies may require twice as much vitamin D as full term babies during the early period of most rapid growth, after which time the requirement should be the same as for babies born at term.

For children between infancy and adolescence a daily allowance of at least 750 cc. of milk together with from 300 to 400 units of vitamin D permits consistently ample retention of calcium and phosphorus. The optimal quantity of vitamin D cannot be stated accurately, though it appears probable that the total quantity needed is neither greater nor less than the amount required for the infant.

For adolescents a need for vitamin D exists, but insufficient data are available to permit an estimate of the quantity required. It seems probable that from 300 to 400 units a day would be satisfactory.

For adults the optimal amount of vitamin D, if a need exists, remains to be determined.

It appears strongly advisable to give vitamin D during pregnancy and lactation. The optimal amount is not known. During lactation the requirement may be greater than at any other period of life and a daily dosage of 800 units or more is suggested, together with an abundant intake of calcium and phosphorus.

CHAPTER XXVII

THE USE OF VITAMIN D PREPARATIONS IN THE PREVENTION AND TREATMENT OF DISEASE

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But for rickets vitamin D would not have been discovered. Its discovery was the secret to rickets; its use is essentially the therapy of that disease. The most direct way to furnish the foundation for intelligent use of vitamin D is to review briefly the sources and requirements of this factor from the point of view of practical therapeutics.

At the present time no less than ten forms of vitamin D are known,[1] but only two have practical importance. One is activated ergosterol of plant origin, in its pure form known as calciferol and given the name of viosterol by the Council on Pharmacy and Chemistry; the other is activated 7-dehydrocholesterol, of animal origin, the form of vitamin D discovered by Waddell[2] and the one which develops in the skin under the action of ultraviolet rays.[3] The short ultraviolet rays of the sun or those derived from artificial sources (such as the quartz mercury vapor lamp and the carbon arc lamp) convert the provitamin in the skin, 7-dehydrocholesterol, into the active form. This is absorbed by the blood. If the vitamin is fed, it is absorbed from the small intestine as a result of the action of bile. In the one case the vitamin formed on the surface of the body finds entrance into the interior through the skin; in the other, ingested ready made, it is absorbed through the intestine. The result is the same.

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[1] Bills, C. E.: The Chemistry of Vitamin D, J. A. M. A. **110**: 2150 (June 25) 1938.

[2] Waddell, James: The Provitamin D of Cholesterol: I. The Antirachitic Efficacy of Irradiated Cholesterol, J. Biol. Chem. **105**: 711 (July) 1934.

[3] Windaus, Adolf; Lettré, H., and Schenck, F.: Ueber das 7-Dehydro-Cholesterin, Ann. d. Chem. **520**: 98, 1935.

The accepted standard unit for expressing the strength of vitamin D, as adopted by the United States Pharmacopeia, is defined as the vitamin D activity of 1 mg. of the international standard solution of irradiated ergosterol, found equal to 0.025 microgram of crystalline vitamin D. This is the international unit accepted as the U. S. P. unit, except that the U. S. P. standard is a reference cod liver oil that has been assayed against the international standard. In stating doses of antirachitic agents, one should think in terms of international units (or U. S. P. units, which are identical [4]), for that is the only way in which the various substances containing vitamin D, which differ from one another so much in bulk, can be reduced to a common denominator. One quart of irradiated milk, for example, contains 135 U. S. P. units, whereas 1 mg. of calciferol contains 400,000.

It is necessary to understand that vitamin D is present in only a few foods ordinarily eaten by us; namely, in milk and some dairy products, in liver and the flesh of certain fish and in eggs. Campion, Henry, Kon and MacKintosh [5] have found that the milk obtained in June from cows kept outdoors contains from 17 to 26 units per quart, a very small amount, whereas the milk of cows kept indoors contains only from 5 to 8 units per quart, practically none. Bechtel and Hoppert [6] reported the content of summer milk as 40 units per quart and of winter milk as 5 units per quart. Apparently pasture feeding has no influence on the vitamin D content of milk, but pasture life in summer does because of exposure of the cow to the sun's rays. The point to realize is that unless special means are taken to enrich the milk with the vitamin, such as feeding irradiated yeast, irradiating the milk itself or adding the vitamin directly, milk is a feeble source of vitamin D. The vitamin in milk is in the butterfat. As expected from the foregoing account, this assays at low values. Average butter is estimated as containing only 80 units per hundred grams, having only about one-hundredth the potency of cod liver oil.

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[4] In this article, wherever units of vitamin D are mentioned, the U. S. P. XI unit is meant.

[5] Campion, J. E.; Henry, K. M.; Kon, S. K., and MacKintosh, James: The Source of Vitamin D in Summer Milk, Biochem. J. **31**: 81 (Jan.) 1937.

[6] Bechtel, H. E., and Hoppert, C. A.: Study of Seasonal Variation of Vitamin D in Normal Cow's Milk, J. Nutrition **11**: 537 (June) 1936.

Mammalian liver contains from 10 to 45 units per hundred grams, again a small quantity.[7] The content of liver is probably also extremely variable; it may be nil. Canned fish, sardines and salmon contain small amounts of vitamin D. The vitamin D of eggs is contained entirely in the yolk. Samples of yolks of February eggs have been reported to contain 140 units of vitamin D, and of June eggs 390 units per hundred grams.[8] Although eggs, like milk, can be improved from the standpoint of vitamin D content if special means to that end are taken, the important point is that as ordinarily marketed they contain too small and at the same time too uncertain an amount of vitamin D to be counted on as a dependable source.

The Eskimos must take extremely large, at times tremendous, quantities of vitamin D in their diet, since through the exigencies of nature they eat largely fish and the fatty flesh of fish-eating animals and birds. Seal flesh is high in vitamin D.[9] The Eskimos owe their protection from rickets and osteomalacia to their diet. Rickets makes its appearance among them as soon as our foods become substituted for theirs.[10] This fact in itself is a cogent indictment of our diets for deficiency in vitamin D. By selection of vitamin D-containing foods (the flesh of certain oily fish, eggs, butter, liver) we also could obtain a moderate supply of vitamin D from food alone, but the diets which we do choose (ordinary milk, fruit, sugar, cereal grains, tubers, green vegetables and muscle meat) must furnish at most a negligible amount of the vitamins. The infant whose diet consists of milk, sugars, fruit juices and cereals during the early months, to be supplemented later with green vegetables, potato, muscle meat and egg, will lack a supply of vitamin D almost entirely unless it is furnished by the summer sun's rays or some specially provided alimentary source.

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[7] de Vaney, G. M., and Munsell, H. E.: Vitamin D Content of Calf, Beef, Lamb and Hog Liver, J. Home Econ. **27:** 240, 1935.

[8] de Vaney, G. M.; Munsell, H. E., and Titus, H. W.: Effect of Sources of Vitamin D on Storage of the Antirachitic Factor in the Egg, Poultry Sc. **12:** 215, 1933.

[9] Rabinowitch, I. M.: Clinical and Other Observations on Canadian Eskimos in Eastern Arctic, Canad. M. A. J. **34:** 487 (May) 1936.

[10] Daniel, E. P., and Munsell, H. E.: Vitamin Content of Foods, Miscellaneous Publication 275, U. S. Department of Agriculture, June 1937. Schastin, N. R., and Petrjajeff, A. T.: Rachitis bei Kindern auf der Insel Kolgujew, Jahrb. f. Kinderh. **140:** 314, 1933. Thomas, W. A.: Health of Carnivorous Race: Study of Eskimo, J. A. M. A. **88:** 1559 (May 14) 1927.

THE ARMAMENTARIUM FOR THE CORRECTION OF VITAMIN D DEFICIENCY

I shall not discuss the relationship of ultraviolet light to vitamin D activation or the treatment of vitamin D deficiency with ultraviolet light. These subjects are dealt with in a separate section by Ethel Luce-Clausen. Instead I shall list the preparations available for the prevention and treatment of vitamin D deficiency by way of the alimentary tract, at the same time giving information of a useful, practical nature about each.

Cod Liver Oil.—The U. S. Pharmacopeia XI requires that cod liver oil shall contain not less than 85 units per gram. As a matter of fact, probably most cod liver oil marketed in this country contains at least 100 units per gram and some of the superior brands two and even four times this official standard. One teaspoonful of cod liver oil, about 4 Gm., supplies about 340 units if the product is U. S. P. minimum standard strength, or about 400 units if the oil is slightly more potent. The vitamin in cod liver oil is probably chiefly activated 7-dehydrocholesterol, the same as the one which is formed in the skin through irradiation.

Cod liver oil is the time-honored remedy for the cure of rickets. Its use goes back into the middle ages.[11] Its advantages are: It is extremely effective in the treatment of rickets (osteomalacia), as was pointed out so clearly years ago by Trousseau;[12] it is universally available; despite assertions to the contrary,[13] it is safe;[14] it contains vitamin A in abundance (about 1,000 units per gram), and its fat is a valuable addition to the diet of many infants. The disadvantages are its lack of concentration, which makes the dose requirement large, and its fishy taste.

It is not practical to attempt to give more than 4 teaspoonfuls, about 1,600 units, daily. Cod liver oil is therefore not potent enough—not enough can be given—to prevent or cure rickets in some premature babies and in especially susceptible full term infants and older children. It is not the best remedy to stop rickets quickly. If more units of vitamin D are required than can easily be given in cod liver oil, it is necessary to turn to the concentrated preparations of vitamin D.

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[11] Guy, R. A.: The History of Cod Liver Oil as a Remedy, Am. J. Dis. Child. **26:** 112 (Aug.) 1923.

[12] Trousseau, Armand: Clinical Medicine, Philadelphia, Lindsay & Blakiston **2:** 734, 1882.

[13] Agduhr, Erid: Untersuchungen über den Einfluss einiger natürlicher Fette und ihrer Komponenten auf tierische Gewebe: der Einfluss verschiedener Komponenten des Dorschlebertrans auf bestimmte Organe, Ztschr. f. Vitaminforsch **5:** 27 (Jan.) 1936.

[14] Cox, W. M., and Roos, A. J.: On the Alleged Toxicity of Cod Liver Oil, Bull. Johns Hopkins Hosp. **54:** 430 (June) 1934.

The fishy taste of cod liver oil virtually precludes its use in the case of the older child and adult. Most infants, however, will take it well and may even develop a liking for it if its administration is begun early and it is given regularly. If it is not given until toward the end of the first year or if its use is allowed to lapse, difficulties in administration will arise. When an infant refuses cod liver oil the trouble usually lies with the mother, who transfers her feelings and prejudices. The ease and success with which cod liver oil may be given to children over 2 years of age when the force of example is brought into play, as in nursery schools, bear witness to the influence of the attitude of adults. One of the obstacles to the use of cod liver oil in the lay mind is the deep-rooted belief that it causes digestive disturbances, especially in hot weather, and that some infants are constitutionally unable to take it at all. Cod liver oil rarely gives rise to digestive disturbances and can be administered in hot weather without any difficulty. However, it must be admitted that some infants cannot take it. In rare instances hypersensitiveness to it may exist.[15] If aspirated it may produce lipoid pneumonia. On account of this danger as well as on account of its low potency other more concentrated forms of vitamin D are to be preferred for premature infants.

Cod liver oil may be given directly from the spoon. It may, however, be floated on orange juice and the two fed together. As much as three teaspoonfuls can be given as a single dose. If the infant does not readily take it, it is wise to turn to some other preparation of vitamin D.

Viosterol in Oil.—Viosterol in oil is ergosterol activated by irradiation or by other methods and dissolved in corn oil or some other bland oil. The United States Pharmacopeia requires that 1 Gm. of viosterol in oil shall contain "at least 10,000 units." In order to prescribe viosterol in oil, it is necessary to know that the vitamin D content of 1 drop is 222 units. There are variations in the quantity of oil delivered by droppers because of variations in the droppers themselves. The manufacturer tries to compensate for these variations by using droppers which will deliver slightly more than the certified amount. Consequently the dose may slightly exceed the calculations. Viosterol in oil is prepared in such a way that it is a hundred times stronger in vitamin D content than standard cod liver oil. Its concentration is a great practical advantage but makes overdoses possible.

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[15] Menagh, F. R.: Etiology and Results of Treatment in Angioneurotic Edema and Urticaria, J. A. M. A. **90:** 668 (March 3) 1928.

Viosterol in oil is tasteless; no difficulty is ever encountered in administering it. In experienced hands it is best dropped directly into the mouth. If difficulty is met, it can be dropped on orange juice in a teaspoon and floated into the mouth. It should never be added to the feeding, lest a part be lost by adhering to the sides of the bottle.

The disadvantage of viosterol, as compared with the fish liver oils, is that it does not supply vitamin A. Viosterol is well adapted for the use of older children and adults.

Before leaving the subject of viosterol it is well to point out that it is now manufactured in two different ways, one by means of ultraviolet irradiation, the other by bombardment with low velocity electrons. Viosterol made by the latter process seems to be the same chemically and biologically as the product of irradiation.[16]

Viosterol (Calciferol) in Propylene Glycol.—This preparation is alleged to be pure vitamin D, prepared from irradiated ergosterol, dissolved in propylene glycol. Its distinctiveness lies in the solvent employed. Because of the solubility of propylene glycol in water, it can be mixed directly with milk. The customary procedure is to drop the dose into the milk. The disadvantage of the preparation is that it does not contain vitamin A. It is standardized so that its potency corresponds exactly with that of viosterol in oil.

Fish Liver Oils Enriched with Viosterol or Some Natural Source of Vitamin D.—To halibut liver oil, which is far richer than cod liver oil in vitamin A but only a few times more potent in D, viosterol or some fish liver oil highly concentrated in D is added in the amount necessary to raise the vitamin D concentration to equal that of viosterol in oil. The object is to obtain a preparation which is concentrated in A as well as D. If viosterol is used as the reinforcing agent, practically the entire D content of the product is viosterol. The vitamin A content of halibut liver oil varies but is standardized at about 50,000 units per gram. The administration should be like that of viosterol in oil.

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[16] (*a*) McQuarrie, Irvine; Thompson, W. H.; Stoesser, A. V., and Rigler, L. G.: The Antirachitic Potency of Ergosterol Activated by Low Velocity Electrons, J. Pediat. **10**: 295 (March) 1937. (*b*) Bills, C. E.; Massengale, O. N.; Imboden, Miriam, and Hall, Helen: The Multiple Nature of the Vitamin D of Fish Oils, J. Nutrition **13**: 435 (April) 1937.

Fish Liver Oils Highly Concentrated in Vitamin D.—Oils from the livers of different species of fish vary greatly in concentration of both vitamin D and vitamin A. Some are rich in A, some in D and some in both. The suborder of fish highest in vitamin D is the percomorphi.[16b] The blue fin tuna heads this group, with the enormous concentration of 40,000 units per gram. What the manufacturers have done in the preparation of these concentrated products is to combine oils from different species in such a way that the final admixture will have the same concentration as viosterol in oil; namely, 10,000 units of vitamin D per gram (1 drop contains 222 units). Percomorph oil is standardized at about 60,000 units of vitamin A per gram, a hundred times the concentration of standard cod liver oil. These fish liver oils have the merit, therefore, of providing D and A in high concentration so that the two can be administered together in doses measured in drops. Naturally the taste is fishy, but the quantity required is very small. Administration should be like that of viosterol in oil.

Irradiated Cholesterol.—This is obtained by the irradiation of cholesterol. The form of vitamin D is activated 7-dehydrocholesterol. Its efficiency in the treatment of rickets has been investigated by Tisdall, Drake and Brown,[17] Hood and Ravitch[18] and, more recently, by Drake, Tisdall and Brown[19] and found to be excellent.

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17. Tisdall, F. F.; Drake, T. G. H., and Brown, A. G.: Irradiated Cholesterol in Cure of Human Rickets; Preliminary Communication, Canad. M. A. J. **32**: 490 (May) 1935.

18. Hood, J. S., and Ravitch, Irene: The Antirachitic Efficiency of Irradiated Cholesterol, J. Pediat. **11**: 521 (Oct.) 1937.

19. Drake, T. G. H.; Tisdall, F. F., and Brown, A. G.: A Comparison of the Antirachitic Effect of Irradiated Cholesterol and Cod Liver Oil, J. Pediat. **9**: 421 (Oct.) 1936.

Miscellaneous Preparations Whose Vitamin D Is Derived from the Fish Liver Oils or Viosterol.—Cod liver oil concentrate is marketed in liquid, capsule or tablet form. Concentrates from the fish liver oils are combined with carotene. Viosterol is furnished in combination with dicalcium phosphate and similar compounds. The dose varies with the preparation as indicated by the label.

Vitamin D Milk.—Milk is the staple ingredient of the diet of the infant and the chief source of calories. The great advantage of placing vitamin D in milk is that it is taken willy-nilly. The disadvantage is that the amount of milk supplying the desired number of calories may not at the same time supply the full vitamin D requirement, which seems to vary with age (growth) rather than with weight.[20] There are three kinds of vitamin D milk.[21]

Irradiated vitamin D milk is produced through the exposure of milk in a thin film for a brief period to the active rays of ultraviolet light from artificial sources. The milk is standardized at 135 units per quart, although some products contain as much as 200 units per quart, as indicated on the label. The potency of ordinary irradiated milk, which is obviously low, cannot be increased greatly by irradiation without the production of an unpleasant taste. The form of vitamin D produced in milk by irradiation is probably activated 7-dehydrocholesterol, as already stated. Irradiated milk is marketed fresh and also in evaporated and dried form. The latter forms are standardized so as to contain 135 units to the reconstituted quart.

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[20] For example, in the study of L. T. Davidson, K. K. Merritt and S. S. Chipman (Prophylaxis of Rickets in Infants with Irradiated Evaporated Milk, Am. J. Dis. Child. **53:** 1 [Jan.] 1937) in which prevention of rickets was attempted by the use of irradiated milk alone, the average intake of milk for the full term infants at the age of 1 month was 396 cc. This amount of milk yielded only 57 units of vitamin D. At the age of 6 months the increased amount of milk taken supplied 114 units. In the case of the premature infants the average amount of milk taken at 1 month was 284 cc. The vitamin supplied by this quantity was only 41 units daily! At 6 months the vitamin D supplied had risen to 103 units daily. The need for vitamin D is greatest during the first four months of life, but during this period the intake of milk was so small that the vitamin D requirement was not met.

[21] For the methods of preparation and standardization of these varieties of activated milk the following references are available: Becker, J. E., and Conn, L.: How the Potency of Vitamin D Milk is Determined, Ninth Annual Report, New York Association of Dairy and Milk Inspectors, 1935, p. 153. Shrader, J. H.: Fortification of Milk with Vitamin D, Arch. Phys. Therapy **15:** 709 (Dec.) 1934. Krauss, W. E., and Bethke, R. M.: New Developments in the Field of Vitamin D Milk, Bimonthly Bulletin, Ohio Agricultural Experiment Station **22:** 3, No. 184, 1937. Official statements from the Council on Foods of the American Medical Association: The Present Status of Vitamin D Milk, J. A. M. A. **108:** 206 (Jan. 16) 1937; Vitamin D Fortified Milk, ibid. **108:** 1515 (May 1) 1937. Vitamin D Milk, Current Comment, ibid. **108:** 1894 (May 29) 1937; Vitamin D Milk Produced by Feeding Cows Irradiated Yeast, ibid. **109:** 1814 (Nov. 27) 1937.

Metabolized vitamin D milk is produced by feeding irradiated yeast to the cow. It is standardized so as to contain not less than 400 units per quart. Its potency in rat units is thus approximately three times that of irradiated milk. The form of vitamin D is irradiated ergosterol, which apparently passes through the cow into the milk unchanged.[22]

Fortified vitamin D milk is produced through the direct addition of a vitamin D–containing concentrate derived from cod liver oil, other fish liver oils, viosterol or fish liver oil emulsions. It is standardized at 400 units per quart. The form of vitamin D in the milk varies naturally with the form in the supplement.

In the case of each of the three varieties the taste is not impaired by the enrichment process, so that the milk is a hidden source of vitamin D and the problem of administration, whether to old or young, is merely that of administration of the milk itself.

Vitamin D Bread.—Bread has been produced containing 460 units in the form of viosterol to the 24 ounce loaf. Six slices would yield about 115 units.

RELATIVE VALUES OF DIFFERENT FORMS OF VITAMIN D

In 1930 the surprising observation was made that viosterol was far less effective, rat unit for rat unit, in curing leg weakness in chickens than was cod liver oil. This observation at once suggested that the two might have different degrees of effectiveness in the human being as well. Quickly investigating this lead, Hess, Lewis and Rivkin[23] reported that the activity of cod liver oil and that of viosterol for the human being were actually different, 1 rat unit of cod liver oil accomplishing the work of 4 of viosterol. In 1931 Hess, Lewis, MacLeod and Thomas[24] reported further that yeast milk, rat unit for rat unit, was more effective in the human being than cod liver oil or viosterol, the efficiency equivalences being in order of 1:1.25:5.2, respectively. In 1933 Hess and Lewis[25] published evidence that for the human being irradiated milk was even more potent than yeast milk when the two were compared with viosterol. These studies were upsetting: Did the infant stand somewhere between the rat and the chicken in relationship to the two forms of vitamin D? Was vitamin D dispersed in milk superior to vitamin D dispersed in oil?

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[22] Bethke, R. M.; Krauss, W. E.; Record, P. R., and Wilder, O. H. M.: The Comparative Antirachitic Efficiency of Vitamin D in Irradiated Milk, Metabolized (Yeast) Milk and Cod Liver Oil, J. Nutrition **11:** 21 (Jan.) 1936.

[23] Hess, A. F.; Lewis, J. M., and Rivkin, Helen: Newer Aspects of the Therapeutics of Viosterol (Irradiated Ergosterol), J. A. M. A. **94:** 1885 (June 14) 1930.

[24] Hess, A. F.; Lewis, J. M.; MacLeod, F. L., and Thomas, B. H.: Antirachitic Potency of the Milk of Cows Fed Irradiated Yeast or Ergosterol, J. A. M. A. **97:** 370 (Aug. 8) 1931.

At least thirty-nine studies have been made in this country and in Canada which bear on these questions. Out of the conflicting and most confusing results only generalizations of a tentative nature are possible.[26] For practical purposes the vitamin D in viosterol may be regarded as being equal to the vitamin D of cod liver oil. If viosterol is inferior to cod liver oil, rat unit for rat unit, and it very likely is, the difference cannot be great or it would have come out more clearly. Vitamin D is more effective dispersed in milk than dissolved in the usual oil menstruums. Milk enriched with vitamin D in the three different ways may be regarded from the practical point of view, rat unit for rat unit, as having equal value. But evidence suggests that the vitamin D, unit for unit, in irradiated milk may be superior to that in metabolized milk. Vitamin D milk having a potency of 400 units per quart will supply enough vitamin D to meet the requirements of the majority of full term infants. On the other hand, it seems fairly clear that irradiated milk has too low a potency (135 units per quart) to be relied on for this purpose. Four hundred units of vitamin D per quart of milk may be regarded as the minimum concentration in that menstruum to be relied on for protection of the infant. This subject has been discussed in great detail by Jeans.[27] In regard to vitamin D in the oil menstruums, the situation is too confused to permit even tentative judgment. It is suggested that, in the case of the fish liver oils and viosterol, for working purposes from 800 to 1,000 units be regarded as the lowest level which it is wise to employ for the infant.

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[25] Hess, A. F., and Lewis, J. M.: An Appraisal of Antirachitics in Terms of Rat and Clinical Units, J. A. M. A. **101:** 181 (July 15) 1933.

[26] The problem is beset with great practical difficulties. The tests must be made on infants, but infants are available, relatively speaking, only in small numbers and some must be set aside for control purposes. Furthermore, infants are not uniform test subjects. They vary in age, diet, racial stock and individual susceptibility to rickets and treatment with vitamin D. In outpatient studies the mothers cannot be relied on to carry out the treatment prescribed. Constant supervision is required. The study of each child must last for weeks (curative) or months (preventive), but the season when the study can be carried out without great liability to error is limited to five months. To make matters still more difficult, the available criteria for the detection of rickets in its early stages are not entirely satisfactory. The early clinical signs of rickets are notoriously deceiving and cannot be relied on. When the rickets becomes advanced, the x-rays furnish an accurate means of recognition, but in the early stages of the disease their use is attended by error of unknown, but probably considerable, magnitude. Finally, the substances to be compared number at least four, which has caused subdivision into groups so small that results are meaningless. Although the complexities of the problem offer excuse, a considerable proportion of the studies made have been badly thought out or carelessly done or carried out on too small or too heterogeneous a collection of infants to make the results significant. In a number of instances the methods of procedure employed have precluded comparison of the results with those of other studies. I am inclined to agree with Jeans that the hope of solution of the problems involved lies in carefully conducted inpatient studies in which the diet and environmental conditions can be controlled and the really accurate methods of measuring vitamin D deficiency (measurements of the calcium and inorganic phosphorus in the blood serum and of the calcium and phosphorus balance) can be employed, but there have been few of these.

It is a fortunate circumstance in deciding on doses of vitamin D that exact knowledge concerning the lower levels of requirement is not necessary because there is at present no reason to believe that amounts of vitamin D well above the minimum levels have any deleterious influence. The rule which the physician should follow in prescribing vitamin D is to administer it in ample enough quantity to cover any possible requirement.

THE PREVENTION OF RICKETS AND INFANTILE TETANY IN THE AVERAGE CASE

The Use of Vitamin D in Oils.—The period of greatest susceptibility to rickets is the first months of life. Whatever antirachitic substance is employed, it is important that full doses be reached early, certainly during the second month. Cod liver oil is taken as a paradigm. In my opinion its administration should be started at the beginning of the third or fourth week, with a dose of one-half teaspoonful (200 units); after two or three days this should be increased to 1 teaspoonful, so that at the end of the third or fourth week the dose is about 400 units. During the fourth or fifth week the dose is raised to 2 teaspoonfuls (about 800 units). According to circumstances this level may be allowed to stand or may be raised during the second month to 3 teaspoonfuls (1,200 units) daily. There is no possible objection to giving 1,200 units of cod liver oil daily if it is well taken and borne. Indeed it is advisable to give this dose if reason exists for thinking the infant particularly liable to rickets. The dose of from 800 to 1,200 units should be given throughout the first year. Eight hundred units should be given throughout the second year.

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[27] Jeans, P. C.: Vitamin D Milk: The Relative Value of Different Varieties of Vitamin D Milk for Infants; A Critical Interpretative Review. J. A. M. A. **106**: 2066 (June 13), 2150 (June 20) 1936.

In most instances difficulties in administration do not occur during the first year but usually do arise some time during the second. When this happens it is advisable to turn to one of the concentrated fish liver oils, viosterol in oil or milk activated with vitamin D.

Cod liver oil, like liquid petrolatum, causes lipoid pneumonia[28] if it runs down the larynx into the lungs or is aspirated after vomiting. It should not be used, therefore, if vomiting occurs frequently. As already pointed out, it should not be given to premature infants if concentrated preparations are available.

The question is often asked whether the use of cod liver oil should be continued during the summer, since the need is less then on account of the wealth of ultraviolet light in the sun's rays at that season. Theoretically, if the infant is well exposed to the summer sun's rays daily, the dose of cod liver oil (or other antirachitic preparation) can be reduced or the administration stopped altogether. Practically, it is good policy to continue through the summer, because interruption often breaks down habit on the part of mother as well as child and makes resumption difficult or impossible. If cod liver oil is under suspicion as the cause of digestive disturbances or is distasteful, it is best to substitute some other preparation of vitamin D.

If it is decided to use a concentrated vitamin D preparation, such as one of the fish liver oils, a fish liver oil enriched with viosterol, viosterol in oil or irradiated cholesterol, the procedure is the same as that outlined; namely, to begin in the third or fourth week and increase the dose rapidly to 800 units. Depending on circumstances, the dose may remain the same or it may be increased to 1,200 units daily. The dose should be maintained for the first two years as described under the heading "Cod Liver Oil." The mode of administration has already been described.

The Use of the Different Forms of Vitamin D Milk.—If milk is employed as the vehicle for vitamin D, the quantity given must always be chosen by the caloric, never by the vitamin D, need. If the quantity is insufficient to supply the vitamin D need, it should be supplemented with vitamin D from some other source. As already indicated, metabolized vitamin D milk and fortified vitamin D milk are potent enough to be relied on to prevent the development of rickets in the majority of full term infants. It is wise, however, especially if the infant is not under close supervision, to supplement with one of the oils containing vitamin D during the first six months. Four hundred units of the oil preparation should be given, beginning at the third or fourth week of life. If irradiated milk with its low unitage is employed, it must certainly be liberally supplemented with some other preparation of vitamin D throughout the first year.

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[28] Goodwin, T. C.: Lipoid Cell Pneumonia, Am. J. Dis. Child. **48**: 309 (Aug.) 1934.

PREVENTIVE MEASURES FOR ESPECIALLY SUSCEPTIBLE INFANTS

For the protection of premature infants and those who are peculiarly susceptible to rickets, the different forms of vitamin D milk may prove insufficient. No reason exists why they should not be used, but liberal supplementation with preparations highly concentrated in vitamin D is imperative. The best course is to treat the infant as if the activated milk did not supply any vitamin D at all. To premature infants it may be necessary to give 1,000, 5,000 or 10,000 units or for a time even more daily in the form of the highly concentrated fish liver oils, viosterol in oil or other vitamin D preparation. In the case of especially susceptible full term infants and older children it is sometimes necessary to exceed 30,000 units daily. The physician discovers that the case is one of special requirement only when the ordinary preventive treatment proves insufficient. The course then is to increase the dose until an adequate therapeutic level is reached. It is wise to allow three weeks to elapse between increases in doses, because that length of time is needed for effects to become fully evident. When an increase becomes indicated it should be made boldly; i. e., from 5,000 to 10,000 units should be given. How to watch in order to make sure that the dose is holding the rachitic tendency in check is discussed later.

HOW TO CURE RICKETS

Amounts of vitamin D which will prevent rickets in a given case will always cure the disease, but often too slowly. If rickets exists, it is best to use large enough doses of vitamin D to end the disease rapidly. When the disease is under control the doses can be lowered. The vitamin D milks are not potent enough to meet the emergency of the cure of rickets, and cod liver oil itself must be abandoned occasionally for this reason.

If cod liver oil is again taken as a paradigm, the curative dose in the ordinary case is 1,200 units (12 Gm., or three teaspoonfuls) daily. This dose in the form of cod liver oil or the highly concentrated fish liver oils and viosterol preparations is sufficient to bring advanced rickets under control within three weeks in the great majority of cases. If larger doses of vitamin D are required, as happens occasionally, it is best to use the concentrated forms of vitamin D. Sometimes, particularly in the case of premature infants, one is forced to give daily from 10,000 to 20,000 units or occasionally even more in order to bring the disease to a complete termination, and in the case of some older children one may be obliged to raise the dose to 60,000 units daily or even to exceed that amount (see treatment of refractory rickets). When the rickets has been stopped, as ordinarily happens in a month's time, the dose of vitamin D can be tapered off to lower levels but often not to the low preventive levels which serve for the average infant. In the case of some children in whom the tendency to rickets is obstinate, large doses of vitamin D may be required indefinitely. In the case of premature infants the need of large doses is usually temporary.

HOW TO TREAT INFANTILE TETANY WITH VITAMIN D

The same rules apply to the vitamin D treatment of infantile tetany as apply to the treatment of rickets with this exception, that during the first days calcium in some form should be administered with the vitamin. The explanation for this advice is that the initial action of vitamin D in some cases is to cause a depression of the serum calcium content at the same time that it causes an elevation in the level of the inorganic phosphorus. The lowering of the serum calcium excites an outbreak of the tetany. This possibility is avoided if calcium is given simultaneously. If calcium chloride is employed it is advisable to administer from 3 to 4 Gm. as an initial dose, followed by 1 Gm. four times a day for two or three days and then 1 Gm. twice a day. The administration of calcium can usually be stopped entirely at the end of a week or ten days, but the vitamin D treatment should be continued.

VITAMIN D IN THE TREATMENT OF REFRACTORY RICKETS

The physician may occasionally encounter a child with rickets which will not yield to vitamin D, even in large doses. Refractory rickets is most often discovered in children above 3 years of age (late rickets). The survival of the rickets into the age period when it ordinarily dies out means either that extraordinarily bad environmental and dietetic conditions keep the disease alive or else that some not understood peculiarity in the child exists which renders the lime salt-depositing mechanism insensitive to the presence of vitamin D. The manifestations, clinical, x-ray, serologic and metabolic, are essentially the same as those in the ordinary infantile form. The diagnosis of refractory rickets must be made by trial and failure of vitamin D therapy. The therapeutic secret in some cases, as discovered through the audacity of Albright, Butler and Bloomberg,[29] is to increase the dose of vitamin D until an effect is produced. In their case (that of a boy of 16 years whose rickets had persisted from infancy in spite of the various orthodox therapeutic procedures) it was necessary to increase the dose to between 1,000,000 and 1,500,000 units daily before healing was induced! Now to maintain the normal state, Albright [30] states, it is necessary to give 150,000 units daily. In a similar case, that of a 7 year old child, presently to be reported by Guild and Wilkins, it has been necessary to raise the dose to 300,000 units daily to make calcification of the skeleton complete. In both cases proof was established that the fault was not failure to absorb vitamin D from the alimentary tract. Probably instances are extremely rare in which such enormous doses of vitamin D are required. Most cases of refractory rickets of the kind under discussion will yield to the administration of 50,000 or 60,000 units daily.

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[29] Albright, Fuller; Butler, A. M., and Bloomberg, Esther: Rickets Resistant to Vitamin D Therapy, Am. J. Dis. Child. **54**: 529 (Sept.) 1937.

[30] Albright, Fuller: Personal communication to the author.

Before the physician concludes that the condition before him is refractory rickets, he must ascertain, first, that it is not the fairly infrequent disease renal hyperparathyroidism (renal osteitis fibrosa, renal rickets; see Park and Eliot[31] for points in differential diagnosis), which is not influenced favorably by vitamin D therapy, and, second, that it is not one of the rare, recently discovered varieties of endogenous rickets, which are by-products of obscure metabolic disturbance and not dependent at all on vitamin D deficiency or cured by vitamin D administration. Instances of endogenous rickets have been described by de Toni,[32] Fanconi,[33] Debré, Marie, Cléret and Messimy[34] and Schier and Stern[35] and probable instances by Ullrich[36] and Schlesinger.[37] The characteristic features are glycosuria with a low blood sugar content (renal diabetes), acidosis due to the presence of an unidentified organic acid, retardation in physical development (dwarfism) and rickets of an osteoporotic type, which may be extreme. Particularly if glycosuria is found, attention should be directed to the possibility that the condition belongs in this curious category.

Probably another form of rickets of endogenous origin has been described by Kaufmann[38] and by Lignac.[39] Disturbance in cystine metabolism, giving rise to cystinuria, and retardation of growth are characteristic features.

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[31] Park, E. A., and Eliot, M. M.: Rickets, in Brennemann, Joseph: Practice of Pediatrics, Hagerstown, Md., W. F. Prior Company, Inc., 1937, vol. 3, chapter 29.

[32] de Toni, G.: Remarks on the Relations Between Renal Rickets (Renal Dwarfism) and Renal Diabetes, Acta Paediat. **16:** 479, 1933.

[33] Fanconi, G.: Der frühinfantile nephrotisch-glykosurische Zwergwuchs mit hypophosphatämischer Rachitis, Jahrb. f. Kinderh. **147:** 299, 1936.

[34] Debré, Robert; Marie, Julien; Cléret, F., and Messimy, R.: Rachitisme tardif coexistant avec une néphrite chronique et une glycosurie, Arch. de méd. d. enf. **37:** 597 (Oct.) 1934.

[35] Schier, A., and Stern, A.: Incurable Rickets, Arch. f. Kinderh. **78:** 176 (June) 1926.

[36] Ullrich, O.: Ueber neuere Behandlungsverfahren der englischen Krankheit im Dienste der allgemeinen Rachitisbekämpfung: Versager der Rachitistherapie und Tier-rachitis, München med. Wchnschr. **76:** 1433 (Aug. 23) 1929.

[37] Schlesinger, Bernard: Renal Dwarfism and Rickets, Proc. Roy. Soc. Med. **25:** 10, 1931.

[38] Kaufmann, E.: III. Konkrementbildung in der Niere, im Nierenbecken und in den ureteren Nephrolithiasis, Nierensteinkrankheit, Spec. path. Anat. **2:** 1107, 1922.

[39] Lignac, G. O. E.: Cystin Metabolism in Children, Deutsches Arch. f klin. Med. **145:** 139 (Oct.) 1924.

In this connection I must call attention also to reports of refractory rickets in which success in treatment was thought to depend on the administration of alkali.[40] In Bornscheuer's case improvement is said to have occurred when the vitamin D treatment was stopped and the patient placed on a diet yielding a large excess of base. In Boyd's case and in that of Stearns and Warweg the rickets treated with vitamin D yielded on the additional administration of sodium bicarbonate.

Finally I would call attention to the fact that in obstructive jaundice vitamin D is not absorbed well from the alimentary tract. Heymann[41] has recently brought forward additional experimental evidence that the bile is essential for the absorption of vitamin D. Under the title "Rachitis Hepatica," Gerstenberger[42] reported two cases of rickets in infants suffering from congenital obliteration of the bile ducts. He considered the rickets refractory to vitamin D. In all probability the disease was ordinary infantile rickets due to failure in absorption of the small quantities of vitamin D administered.[43]

THE PRODUCTION OF RAPID HEALING

Occasionally it is important to effect a rapid cure of rickets. For example, in "thoracic rickets," described by Park and Howland,[44] the weakness of the thorax menaces life. Under such circumstances it is incumbent on the physician to supply rigidity to the thoracic bellows as quickly as possible. In the case of a baby aged 8 months suffering from advanced rickets, one can give daily doses of 50,000 units of vitamin D. When the healing begins, as can be detected by means of roentgenograms or determinations of the calcium and inorganic phosphorus, the dose should be reduced. Harnapp,[45] Bischoff,[46] and Schirmer[47] have given infants single doses of 600,000 units without bad effect. After a single dose the vitamin probably remains in the body for a number of weeks.[48] I do not recommend the single dose method. The diet should contain ample calcium and phosphorus.

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[40] Boyd, J. D.: Endogenous Rickets, Proc. Soc. Exper. Biol. & Med. **26**: 181, 1928. Bornscheuer, P.: Ein Fall von endogener gegen D-vitamin und Licht resistenter perennierender Rachitis, Ztschr. f. Kinderh. **51**: 56, 1931. Stearns, Genevieve, and Warweg, Edna: Studies of Phosphorus of Blood, Phosphorus Partition in Whole Blood and in Serum and Serum Calcium and Plasma Phosphatase During Healing of Late Rickets, Am. J. Dis. Child. **49**: 79 (Jan.) 1935.

[41] Heymann, Walter: Metabolism and Mode of Action of Vitamin D.: IV. Importance of Bile in the Absorption and Excretion of Vitamin D, J. Biol. Chem. **122**: 249 (Dec.) 1937.

[42] Gerstenberger, H. J.: Rachitis hepatica, Monatschr. f. Kinderh. **56**: 217, 1933.

[43] Thoenes, F., and Gruson, W.: Zur Frage der "Rachitis hepatica," Monatschr. f. Kinderh. **67**: 134, 1936.

[44] Park, E. A., and Howland, J.: The Dangers to Life of Severe Involvement of the Thorax in Rickets, Bull. Johns Hopkins Hosp. **32**: 362 (April) 1921.

HOW TO GAGE THE SUCCESS OF VITAMIN D THERAPY

One can assume that preventive treatment is succeeding if the signs of rickets do not make their appearance in the roentgenogram of the radius and ulna and—provided the infant is full term—if the level of the calcium of the serum remains above 9 mg. and that of the inorganic phosphorus above 5 mg. per hundred cubic centimeters. Rickets can develop in the premature baby even though the levels of calcium and inorganic phosphorus are within the range accepted as normal (calcium 10 mg. and inorganic phosphorus 5 mg. per hundred cubic centimeters of blood). Periodic estimations, at monthly or two-monthly intervals, of the calcium and inorganic phosphorus in the blood serum and x-ray examinations are the most reliable means of determining that rickets is not developing. However, for all practical purposes one can be sufficiently certain that the preventive treatment is successful if clinical evidences of rickets do not make their appearance.

If the problem is one of cure, the condition ordinary rickets and the dose of vitamin D about 1,200 units, the first indication of therapeutic effect will be a rise in the level of inorganic phosphorus in the serum. This begins about ten days after treatment has been begun. About three weeks after the beginning of treatment the shadows of fresh deposits of lime salts make their appearance in the roentgenogram, often in the form of a transverse line, usually broken, crossing the transparent cartilage just beyond the end of the shaft. As treatment continues, this line of deposits becomes thicker and more dense. Finally the gap between it and the end of the shaft becomes completely filled with lime salts. The filling in process may take two months. The pattern of the rickets may last for a very long time after the disease has completely stopped.

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[45] (*a*) Harnapp, G. O.: Zur Pathogenese der Spasmophilie, Behandlungsversuche mit A. T. 10, Monatschr. f. Kinderh. **63**: 262, 1935; (*b*) Rachitisbehandlung durch einmalige Verabfolgung von Vitamin D_2, ibid. **66**: 318, 1936.

[46] Bischoff, H.: Rachitisbehandlung mit konzentrierten Vigantol, Jahrb. f. Kinderh. **150**: 2, 1937.

[47] Schirmer, R.: Rachitisbehandlung mit einmaliger Vitamingabe, Monatschr. f. Kinderh. **68**: 269, 1937.

[48] Heymann, Walter: Metabolism and Mode of Action of Vitamin D: II. Storage of Vitamin D in Different Tissues in Vivo, J. Biol. Chem. **118**: 371 (April) 1937.

This is the ordinary course of events, but if larger doses of vitamin D are given the changes in the blood and the roentgenograms can be more rapid. For example, after very large single doses of vitamin D Harnapp,[45b] Bischoff[46] and Schirmer[47] reported rises in the serum calcium and phosphorus content and signs of fresh depositions of lime salts in the roentgenograms as early as the fifth day.

It is difficult to be sure of the healing effect of treatment from clinical examination alone, for recession of the deformities is gradual. Perhaps the most obvious clinical indication of successful treatment is the improvement in muscular function. The child begins to make efforts to walk or to sit up. He seems stronger and more active.

If the treatment is adequate the improvement will be steadily progressive; if inadequate, either no improvement or improvement which presently stops will occur.

VITAMIN D IN THE TREATMENT OF OTHER CONDITIONS

The use of vitamin D has been extended to a variety of diseases far removed from rickets. Apparently the basis of its use for some of the diseases has been the belief that its toxic effects are beneficial. The justification is entirely empiric.

I mention the diseases in question and give references to the literature but cannot pass judgment. I remark merely that the evidence offered in favor of the therapeusis in some instances seems rather slim.

Rappaport and Reed[49] have reported favorable effects from massive doses of vitamin D for hay fever; Dreyer and Reed,[50] Wyatt, Hicks and Thompson,[51]

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[49] Rappaport, B. Z., and Reed, C. I.: Viosterol of High Potency in Seasonal Hay Fever and Related Conditions, J. A. M. A. **101:** 105 (July 8) 1933.

[50] Dreyer, Irving, and Reed, C. I.: The Treatment of Arthritis with Massive Doses of Vitamin D, Arch. Phys. Therapy **16:** 537 (Sept.) 1935.

[51] Wyatt, B. L.; Hicks, R. A., and Thompson, H. E.: Massive Doses of Vitamin D in the Treatment of Proliferative Arthritis, Ann. Int. Med. **10:** 534 (Oct.) 1936.

Livingston,[52] Vrtiak and Lang[53] and Farley,[54] for chronic arthritis. The dose ranged from 200,000 to 1,000,000 units (in one instance) given daily over periods of weeks or months. Toomey[55] has suggested vitamin D therapy as a possible means of increasing resistance to infantile paralysis. Krafka[56] and more recently Ceder and Zon[57] (the latter authors used massive doses) have reported striking effects in psoriasis. Weld[58] has reported favorable action in increased capillary permeability, and McNealy, Shapiro and Melnick[59] and Johnston,[60] in checking of the bleeding tendency in cases of obstructive jaundice. Richardson[61] stated that vitamin D given during pregnancy may reduce the duration of labor and the loss of blood. Theobald[62] has found it useful in treating the toxemia of pregnancy.

EVIDENCE THAT DOSE IS EXCESSIVE

The idea that vitamin D in the form of irradiated ergosterol is an extremely toxic substance was derived from the use of the old German preparation vigantol, in the wake of which metastatic calcification was observed by a variety of investigators.[63]

In the treatment of chronic arthritis and psoriasis enormous doses of vitamin D have been employed. From these experiences, which have been limited to adults, it is possible to obtain an idea of the symptoms produced by overdoses. Wyatt, Hicks and Thompson,[51] who limited the dose to 300,000 units daily, reported in one case violent persistent nausea, intense headache and profuse sweating and in another severe diarrhea, loss of appetite and slight headache. Vrtiak and Lang[53] used from 150,000 to 250,000 units daily over periods of months. They reported that in all their patients nausea developed and in some nocturia and frequent micturition. Farley,[54] who in most instances administered 200,000 units but in cases of obstinate arthritis increased the dose to 300,000, 600,000 units and even (in one case) 1,000,000 units daily, noted nausea and increased micturition. He stated that toxic symptoms seldom developed if the dose remained under 400,000 units. Cedar and Zon[57] used doses of 300,000 to 400,000 units daily. They noted anorexia, nausea, malaise and urinary frequency after 10 to 12 weeks of treatment. A progressive hypercalcemia developed, the maximum range varying from 12 to 16 mg. per 100 cc. of serum. In Guild and Wilkins' 7 year old child with refractory rickets receiving 300,000 units, the symptoms indicating that the proper dose had been exceeded were loss of appetite, pallor and lassitude.

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[52] Livingston, S. K.: Vitamin D and Fever Therapy in Chronic Arthritis, Arch. Phys. Therapy **17:** 704 (Nov.) 1936.

[53] Vrtiak, E. G., and Lang, R. S.: Observations on the Treatment of Chronic Arthritis with Vitamin D, J. A. M. A. **106:** 1162 (April 4) 1936.

[54] Farley, R. T.: Management of Arthritis, Illinois M. J. **71:** 74 (Jan.) 1937.

[55] Toomey, J. A.: Ingestion of Vitamins A, B, C and D and Poliomyelitis, Am. J. Dis. Child. **53:** 1202 (May) 1937.

[56] Krafka, Joseph, Jr.: Simple Treatment for Psoriasis, J. Lab. & Clin. Med. **21:** 1147 (Aug.) 1936.

[57] Ceder, E. T., and Zon, Leo: Treatment of Psoriasis with Massive Doses of Crystalline Vitamin D and Irradiated Ergosterol: A Preliminary Report, Pub. Health Rep. **52:** 1580 (Nov. 5) 1937.

[58] Weld, C. B.: A Capillary Resistance Test and Its Relation to Vitamins C and D, J. Pediat. **9:** 226 (Aug.) 1936.

[59] McNealy, R. W.; Shapiro, P. F., and Melnick, P.: Effect of Viosterol in Jaundice, Surg., Gynec. & Obst. **60:** 785 (April) 1935.

[60] Johnston, L. B.: Use of Massive Doses of Viosterol to Reduce Bleeding Time in Obstructive Jaundice: Report of Three Cases, J. Med. **18:** 235 (July) 1937.

[61] Richardson, G. C.: Viosterol in Pregnancy: Review of 300 Cases. Illinois M. J. **65:** 367 (April) 1934.

[62] Theobald, G. W.: Effect of Calcium and Vitamins A and D on Incidence of Pregnancy Toxaemia, Lancet **1:** 1397 (June 12) 1937.

[63] Bills, C. E.: Physiology of the Serols, Including Vitamin D, Physiol. Rev. **15:** 1 (Jan.) 1935.

If the dose is high, the patient a child, and the condition rickets, the physician should examine the urine daily, looking in particular for calcium casts, such as Albright and Bloomberg[64] have described in cases of hyperparathyroidism. The casts will not be found if the urine is sufficiently acid. He should also keep close track of the levels of the calcium and inorganic phosphorus in the blood serum. An elevation of serum calcium above 12 mg. per hundred cubic centimeters is the danger signal. In connection with one of their cases Albright, Butler and Bloomberg write:[65] "The calcium content of the serum rose to 12.7 mg. and the phosphorus content to 3.5 mg., and the phosphatase content fell to 13.5 Bodansky units per hundred cubic centimeters. This increase in the serum calcium and phosphorus was fortunately noted at once, so the dose was lowered and was eventually stopped for a short time. At the height of the rise in the blood values the urine was loaded with calcium and contained many calcium casts such as have been observed in cases of hyperparathyroidism." With the rise in the serum calcium a rise in inorganic phosphorus takes place. However, the inorganic phosphorus may not reach even the normal level. If the excessive vitamin D therapy is persisted in, undoubtedly the inorganic phosphorus level as well as that of the calcium will become elevated and metastatic calcification will occur, as is regularly the case in animals subjected to toxic doses of vitamin D.[66] The physician will also gain considerable assistance by taking x-ray films of the radius and ulna in order to determine if lime salts are depositing in the rachitic zone. If the film reveals shadows which indicate healing, he learns the important fact that the vitamin has begun to act and that the calcium and phosphorus levels have been affected. The signs of healing in the roentgenogram do not mean that the dose of vitamin D should be lowered but warn the physician that toxic effects are about to become possible. How often the physician should examine the blood and have roentgenograms taken depends on the size of the dose and the age and size of the child. If extremely large doses, for example, 100,000 units or more are given, examinations of the blood ought to be made weekly or, in critical situations, at semiweekly intervals and x-ray examinations of the bones at weekly or bi-weekly intervals.

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[64] Albright, Fuller, and Bloomberg, Esther: Hyperparathyroidism and Renal Disease, with a Note as to the Formation of Calcium Casts in This Disease, Tr. Am. A. Genito-Urin. Surgeons **27:** 195, 1934.

[65] Albright, F.; Butler, A. M., and Bloomberg, E.: Rickets Resistant to Vitamin D Therapy, Am. J. Dis. Child. **54:** 529, 1937.

The physician should be on his guard for signs of toxicity in the case of the child if the daily dose exceeds 50,000 units. If the patient is a very small infant, e. g., a premature infant, he should watch with care even if the dose is 20,000 or 30,000 units. Toxic manifestations will not occur immediately but only after a period of two or three weeks. The rule probably holds that toxic manifestations need not be expected so long as the rickets remains entirely unaffected. Hence the usefulness of x-ray films of the radius and ulna as a safeguard in treatment.

With the adult suffering from arthritis, etc., the physician ought to exercise great caution in venturing into the unknown with daily doses of 200,000 units or more. I had always supposed that the development of toxic symptoms was dependent on the effects of vitamin D on the calcium and phosphorus metabolism. Reed,[66a] however, who has studied this problem specially, assures me that such is not the case. "The prevalent idea that the toxicity due to hypervitaminosis D depends on the production of hypercalcemia has been shown by a number of workers to be incorrect. It is true that hypercalcemia and toxicity occur together quite commonly. It is also true that measures which will reduce hypercalcemia will, to some extent, alleviate the symptoms at least temporarily. . . . However, Reed and Thacker showed that in experimental animals severe symptoms of intoxication and even death might result without any hypercalcemia. On the other hand, pronounced hypercalcemia has been found in both dogs and human subjects to persist for weeks and even months without any evidence of the symptoms complex of toxicity." In summary it seems best to regard a rise in the serum calcium to or beyond 12 mg. per one hundred cubic centimeters as a toxic manifestation of vitamin D but not as one necessarily fraught in itself with serious consequences. Here again, however, the rise is a most useful danger signal. Apparently the indications for the reduction or cessation of vitamin D treatment which have been employed by those who have used these enormous doses of the drug have been the clinical signs of illness in the patient himself, i. e., if the vitamin seems to be making the patient sufficiently sick.

[66a] Reed, C. I.: Personal communication.

The following distinction is in order. In rickets (osteomalacia) the object is to restore normal calcification to the skeleton. The physician does not need to induce toxic action to bring this to pass and seeks to avoid even an approach to it. In the diseases like chronic arthritis, psoriasis, etc., which do not depend, as far as is known, on disturbances in the calcium-phosphorus metabolism, the object is to obtain a therapeutic action of a totally unknown nature which is probably quite distinct from the physiological action resulting in the deposition of lime salts in the bones and extends close to, if it does not actually lie within, the region of toxic activity. Accordingly, the obligation of the physician in the treatment of the conditions under discussion appears to be, not necessarily to avoid basis levels of the vitamin but rather to make sure that all toxic action which might have injurious effects on the patient is avoided. The reader should be cautioned that the use of vitamin D in the treatment of the above conditions is still on an experimental basis. The future may show that the possible dangers are not justified by the results obtained.

VITAMIN D AND THE TEETH

It is certain that rickets causes enamel hypoplasia.[67] This condition can be prevented by the prevention of rickets. Evidence is accumulating that administration of vitamin D during childhood, particularly in the early years, reduces the incidence of dental caries.[68] The permanent teeth are in process of development from birth during the entire period of growth. The possible influence of vitamin D in the protection of the teeth from hypoplastic deformities and caries is a sufficient reason for the continuation of the vitamin D therapy throughout child life.

THE VITAMIN D REQUIREMENT AFTER THE SECOND YEAR

The reason usually given to explain the particular susceptibility of the infant to rickets is rapid growth, but this explanation really has no meaning. The older child is occasionally subject to rickets ("late rickets"), but the adult never has osteomalacia (rickets after growth has ended) unless under extreme provocative conditions. It is not understood why the child and adult are so insusceptible. The adult rat is far more resistant to rickets-producing diets than the young animal, but even the young rat does not appear to need vitamin D if the diet is perfectly constituted. If, however, deficiencies in the calcium and phosphorus are sufficiently great, vitamin D may exert a striking rectifying effect. It is easy to demonstrate the protecting influence of vitamin D on the skeleton of the pregnant rat. These experiences can probably be carried over in principle to the human adult and to the older child. If the diet is excellent—this includes the supply of calcium and phosphorus—either no vitamin D is required or a very small quantity suffices. If, on the other hand, the supply of calcium and phosphorus is at fault, vitamin D will exert a helpful influence.

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[67] Eliot, M. M.; Souther, S. P.; Anderson, B. A., and Arnim, Sumter: A Study of the Teeth of a Group of School Children Previously Examined for Rickets, Am. J. Dis. Child. **46:** 458 (Aug.) 1933.

[68] Agnew, M. C.; Agnew, R. C., and Tisdall, F. F.: The Production and Prevention of Dental Caries, J. Am. Dent. A. **20:** 193 (Feb.) 1933. Committee for the Investigation of Dental Disease: The Influence of Diet on Caries in Children's Teeth, Medical Research Council, Special Report Series No. 211, London, His Majesty's Stationery Office, 1936.

A large proportion of children and adults must receive a modicum of vitamin D in their food. This modicum, which would be totally inadequate for the infant, may be significant for the adult because of his small requirement. The facts in this regard simply are not known. On the other hand, great numbers of people receive no vitamin D or virtually none from their food. A large proportion of children and adults must obtain liberal supplies of vitamin D from exposure to the sun in the summer, late spring and early fall months, when the active ultraviolet rays are in abundance and this vitamin is probably stored for weeks in the body.[48] Others, particularly those confined indoors or to the hearts of cities, derive little or no vitamin D from this source. Moreover, large groups of persons are denied the sun's rays the year round because of their occupations, e. g., miners, night workers and indoor workers in general, or because of personal habits or necessities (invalids). These are the facts.

In the case of the child the physician should be certain that the administration of vitamin D is not stopped at the end of the second year. It is undoubtedly more important for the child to receive it during the third than during the fourth year and during the fourth than during the fifth. The need of the child for the vitamin probably declines with increasing age. Nevertheless I recommend that it be given throughout the growing period. A sufficient reason for this advice is the possibility that the administration of the vitamin may aid in improving the durability of the teeth.

In the case of the adult the course is not so clear. Probably in most cases in which a physician has the opportunity to advise, the patient does not require vitamin D from an additional source and would not feel different or actually be in better health if he received it. On the other hand, in many instances the patient would probably be benefited, perhaps greatly, by receiving it. It seems a sound policy to advise vitamin D for (1) all patients who by force of circumstances are deprived of the opportunity of obtaining the vitamin from the sun (night workers, factory workers and persons confined indoors, in particular invalids), (2) for all persons whose diet is lacking in milk, the sole valuable source of calcium, or is generally poor, (3) for the aged, particularly when they are limited as to diet and confined indoors, (4) for persons suffering from fractures or having had operations on bones and (5) for all pregnant women, no matter what the diet or the season. With regard to pregnancy, evidence which cannot be disregarded exists that vitamin D is a safeguard both to mother and to infant. The physician should unfailingly see that every woman during pregnancy receives vitamin D.

I want to point out that it would be of undoubted benefit if the habit of taking vitamin D in some form were universal. If our food were like that of the Eskimo, with vitamin D a regular ingredient, rickets would disappear almost entirely, the state of the teeth might undergo improvement and the level of health of many persons might be raised. I believe that the physician ought to extend the use of vitamin D wherever he is able, in particular during the winter months, even if he is unable to measure benefit in individual cases.

As for the preparation of vitamin adapted for the adult and child, cod liver oil is not especially suitable, but the other sources are, the vitamin D milks, the preparations of viosterol in oil and the highly concentrated fish liver oils. For the pregnant woman I suggest a daily dose of 800 units and for the child a dose of from 300 to 400 units. I presume that for the average adult a dose of 135 units daily, such as would be supplied by a quart of irradiated milk or six slices of vitamin D bread, would be more than ample, but I have no means of even guessing what the amount should be.

Before leaving this subject I wish to make two practical comments. First, since the adequacy of all diets in vitamin A for older children and adults has not been satisfactorily established, I suggest that the preparations which combine A and D be chosen in preference to those which contain D alone. Vitamins A and D go together (fat-soluble) and may well be administered together. The concentrated fish liver oils, which are rich in vitamin A as well as D, are to be preferred to the viosterol group for this reason. Second, since older children and adults often take very little milk, fortified and metabolized vitamin D milks are to be preferred as sources of vitamin D to irradiated milk. One glass of irradiated milk, which is as much milk as many adults take in a day, would yield only 33 units of vitamin D. In the light of the previous comment, fortified milk is superior if the addition of the fish liver oil concentrate increases the content of vitamin A as well as that of vitamin D.

RELATIVE COST OF THE DIFFERENT PREPARATIONS OF VITAMIN D

The only reasonable way to compute the relative values of the different preparations of vitamin D is per unit of the contained vitamin. On this basis cod liver oil is the cheapest. It is interesting that some of the cod liver oils having the higher unitage, though costing more per volume, are actually the most economical. For example, at a local inquiry it was found that the cod liver oil with the highest unitage (350 units per gram) sold at 90 cents a pint; its calculated cost per 1,500 units was eight tenths of a cent. Oil selling at 50 cents a pint and having only the required 85 units per gram cost twice as much per 1,500 units, namely, 1.9 cents. The oils depending on viosterol for their enrichment in vitamin D and viosterol itself cost in general twice as much as the concentrated fish liver oils. The fish liver oils highly concentrated in vitamin D are as economical sources as many of the poorer brands of cod liver oil. From the standpoint of unitage, irradiated milk is about one third as potent as metabolized or reinforced milk. In the case of each of the vitamin D milks the enrichment process usually adds 1 cent a quart, although some dairies have not increased the price and vitamin D evaporated milk sells at the same price as ordinary evaporated milk.

CHAPTER XXVIII

PHYSICAL ASPECTS OF ULTRAVIOLET RADIATION IN VITAMIN D THERAPY

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In the present paper an attempt is made to confine the discussion to the physical aspects of ultraviolet radiation in the relation to the production of vitamin D and the healing of rickets.

Modern ultraviolet therapy has an interesting historical background. Following the observations of Huldschinsky, who in 1919 cured rickets in children by means of rays from the mercury quartz lamp, advancements have been rapid. It was shown in turn by various investigators that irradiation would produce in foods an antirachitic effect, that this induced property was destroyed by excessive irradiation, that the activatable substances in foods were ergosterol and a closely related compound associated with cholesterol and, to some extent, probably other sterols.

A particularly valuable tool in the development of this knowledge has been the spectroscope. Considerable information has been accumulated about the spectroscopic properties of vitamin D and related compounds. It is well known that every chemical compound has a characteristic absorption spectrum. Heilbron and his collaborators [1] reported that the ultraviolet absorption spectrum of cholesterol has only a general absorption, which means that it has no bands of selective absorption. They reported also that cholesterol ordinarily contains a small quantity of a foreign substance that has well defined absorption bands at 2,690, 2,800 and 2,930 angstroms, respectively. This substance appears to be a precursor of vitamin D. It disappears on irradiation and the appearance of antirachitic potency. Unfortunately the ultraviolet absorption bands, if any, of vitamin D are not sufficiently pronounced and sufficiently different from that of ergosterol to make possible an exact identification of the antirachitic substance.

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[1] Heilbron, I. M.; Kamm, E. D., and Morton, R. A.: The Absorpton Spectrum of Cholesterol and Its Biological Significance with Reference to Vitamin D, Biochem. J. **21**: 78, 1927. Morton, Heilbron and Kamm, id., On Ergosterol, J. Chem. Soc. London **268**: 2000 (Aug.) 1927.

In chart 1 (see additional data [2] in chart 2) is shown the ultraviolet absorption spectrum of exceptionally pure ergosterol, dissolved in optically pure 95 per cent alcohol (concentration, 1 Gm. per liter) before and after irradiation with a quartz mercury vapor lamp, as described by Bills and his collaborators.[3] The sharp absorption maximums of the nonirradiated oil at 2,700, 2,820 and 2,935 angstroms (270 millimicrons, mμ in the charts), respectively, are to be noted.

Starting with a nonirradiated stock solution of ergosterol, having no antirachitic activity, Bills and his collaborators [3] found that, although no new absorption bands occurred, after seven and a half minutes' irradiation the animal tests revealed the development of an enormous antirachitic potency, 150,000 times that of average cod liver oil. After an exposure of twenty-two and a half minutes the potency increased to 250,000 times that of average cod liver oil. After an irradiation of thirty minutes the maximum at 2,935 angstroms had disappeared; the maximum at 2,820 angstroms was still conspicuous; the maximum at 2,700 angstroms (chart 1) was decidedly distorted, showing the development of a new band at 2,520 angstroms, and the antirachitic potency had dropped to 200,000 times that of the cod liver oil used as a standard. After three hours' irradiation, when the new absorption band at 2,480 angstroms had developed to its maximum intensity, the antirachitic potency had almost vanished. Continuing the irradiation for from four to fifteen hours had but little effect on the new absorption spectrum, which has a maximum at 2,480 angstroms. The antirachitic potency could not be restored, showing that the new photochemical product is not vitamin D, for its appearance coincides with the destruction of antirachitic potency. As a result of these biologic tests with more than 7,000 rats, Bills and his collaborators concluded that the spectroscope cannot replace the biologic method

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2. Bourdillon, R. B.; Fischmann, C.; Jenkins, R. G. C., and Webster, T. A.: The Absorption Spectrum of Vitamin D, Proc. Roy. Soc. London, Series B **104**: 561, 1929. van Wijk, A.; Reerink, E. H., and Mörikofer, W.: Solar Radiation and Vitamin D, Strahlentherapie **39**: 3, 1930.

3. Bills, C. E.; Honeywell, Edna M., and Cox, W. M., Jr.: Quantitative Biophysical Studies on the Activation of Ergosterol, J. Biol. Chem. **80**: 557 (Dec.) 1928

in studying the formation of vitamin D because the wavelengths absorbed by vitamin D and by which it is destroyed apparently lie in the same spectral region as the wavelengths which activate ergosterol.

According to Morton, Heilbron and Kamm[1] the destruction of vitamin D is the result of excessive irradiation (though not exclusively) by wavelengths shorter than about 2,700 angstroms. Reerink and Van Wijk[4] report that irradiation of ergosterol with wavelength 2,537 angstroms gives rise to a series of reaction products that differ from that caused by irradiation with wavelengths longer than 2,750 angstroms. By excluding wavelengths shorter than 2,750 angstroms it was possible to convert 60 per cent of the ergosterol into vitamin D before the secondary reaction (destruction of vitamin D) became important. When the wavelengths 2,537 and 2,654 angstroms were included, only a much smaller amount of ergosterol could be converted into vitamin D before destructive action interfered.

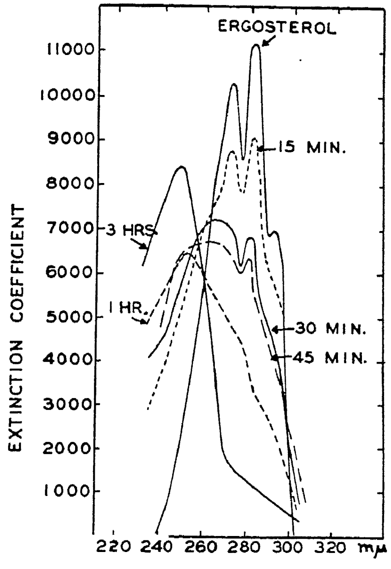

Chart 1.—Effect of irradiation for different lengths of time on the ultraviolet absorption spectrum of ergosterol.

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[4] Reerink, E. H., and van Wijk, A.: The Vitamin D Problem: I. The Photochemical Reactions of Ergosterol, Biochem. J. **23**: 1294, 1929.

Using monochromatic radiation, Marshall and Knudson[5] reported that the highest concentration of vitamin D which could be produced by direct irradiation of ergosterol was 35 per cent. They also report that vitamin D is destroyed by radiation of the same wavelengths that produce it.

No investigations appear to have been made to determine whether, after destruction of vitamin D by the shorter ultraviolet wavelengths (from 2,480 to 2,650), the antirachitic activity can be restored by irradiation with the photochemically effective wavelengths at 2,894 to 2,967 angstroms respectively. In this connection, attention is called to an interesting photochemical reaction that occurs in glass, in which the same wavelength can restore or destroy a chemical constituent, depending on the wavelengths (shorter or longer) used in the previous irradiation.[6]

Early in these investigations Hess[7] reported that rats fed on untreated cadaver and calf skin developed rickets, whereas all rats that received irradiated skin were protected against rickets. The logical deduction seemed to be that the antirachitic effect was brought about by activation of the cholesterol (and ergosterol) in the superficial layer of the skin, just as crystalline cholesterol can be activated by irradiation. More significant, however, seems to be his report, even at this early date, that crystalline cholesterol, after prolonged irradiation, gradually became inactive, and eventually became inert in its effect on rickets.

In view of the foregoing results, even though no ill effects seem to have been reported (and an excessive exposure is limited by skin tolerance as manifested by the erythemal reaction), the question arises as to the desirability of conducting ultraviolet therapy with lamps emitting an excessive amount of ultraviolet radiation of wavelengths shorter than 2,800 angstroms. For example, in the so-called cold quartz type of mercury vapor lamp, more than 95 per cent of the biologically effective radiation that is emitted is of the wavelength 2,537 angstroms (chart 4). These short wavelengths have a specific germicidal action (chart 2) and, hence, are useful in dermatology. Nevertheless it is a question whether rays of such short wavelengths should be used in general ultraviolet therapy. In view of this uncertainty, the Council on Physical Therapy does not accept, for home use without the direction of a physician, lamps that emit an appreciable amount of ultraviolet radiation of wavelengths shorter than 2,800 angstroms.

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[5] Marshall, A. L., and Knudson, A.: Formation of Vitamin D in Ergosterol by Monochromatic Radiation, J. Am. Chem. Soc. **52**: 2304 (May) 1930.

[6] Coblentz, W. W., and Stair, R.: Ultraviolet Transmission Changes in Glass as a Function of the Wavelength of the Radiation Stimulus, J Res. Nat. Bur. Stds. **13**: 773 (Dec.) 1934.

[7] Hess, A. F.: The Antirachitic Activation of Foods and of Cholesterol by Ultraviolet Irradiation, J. A. M. A. **84**: 1910-1913 (June 20) 1925.

Because of the shortcomings of the spectroscopic examination of provitamin D materials, recent investigations on this subject are based principally on the biologic effects in curing rickets. It was early found that different antirachitic substances may not have equivalent values when tested on different species of animals. In view of the great differences in antirachitic action of substances naturally endowed with vitamin D, and of substances antirachitically activated by ultraviolet radiation, as determined biologically on rats and chicks, the question naturally arises as to the effect that may be expected in prevention or healing of rickets in children, either by feeding activated food or by ultraviolet irradiation.

Of especial importance is the question of the intensity of the source of ultraviolet radiation used. The Council on Physical Therapy has had to consider the acceptability of lamps that emitted ultraviolet radiation of so low an intensity that it would have required an exposure of from fifteen to thirty hours or more to produce a threshold erythema (M. P. E.). Yet it was claimed that such lamps effected healing of rickets in rats and chicks.

It was noted that these lamps emitted a large amount of infra-red radiation and the question arose regarding the effect of heat (the temperature of enclosure) on antirachitic action, about which there seems to be a great dearth of information. In this connection the experiments of Ludwig and Ries[8] are of especial interest. New-born rats were reared in especially constructed enclosures, covered with red, blue and clear glass, and exposed (Bern, Switzerland, high elevation, clear atmosphere) for 200 or more days. Throughout the test these experimenters observed the greatest growth under red glass, which they ascribe to the stimulation of the "growth vitamin" by red and infra-red rays, as distinguished from vitamin D stimulation by ultraviolet rays.

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[8] Ludwig, F., and Ries, J. V.: Effect of Ultraviolet and Infra-Red on Growth, Strahlentherapie **39:** 485, 1931.

From the foregoing discussion, and brief references to investigations of vitamin D, it may be noted that the use of ultraviolet radiation involves a number of very complex, and little understood, biochemical and biophysical phenomena, some of the latter of which will now be described, and their relation to phototherapy will be indicated.

THE SPECTRAL RANGE OF ANTIRACHITIC AND ERYTHEMAL REACTION

The earliest experiments on the spectral range of antirachitic and erythemal reaction were confined to the spectral activation of irradiated foods, which, in turn, were fed to rachitic animals to determine the antirachitic effectiveness of the irradiated material. Thus Hess and his collaborators[9] found that cholesterol, after exposure to homogeneous radiation of wavelengths 2,804 and 3,024 angstroms, had a marked healing effect in rachitic rats, whereas the emission line of the mercury arc at 3,132 angstroms had only a slight or no effect on cholesterol, as determined by its power to heal rickets.

Among the earliest direct determinations of the (antirachitic) healing power of different wavelengths of homogeneous radiations are the observations of Sonne and Rekling,[10] who found that the strong emission lines of the mercury arc at 2,537, 2,650, 2,804, 2,967 and 3,024 angstroms had an antirachitic action, whereas wavelengths at 2,400 and 2,480 angstroms had only a slight effect; the emission line at 3,132 angstroms had a doubtful effect, and the line at 3,663 angstroms had no curative effect at all.

Using filters to limit the spectral range, Maughan[11] found that the emission line of the mercury arc at 2,967 angstroms was the most important, and the second in importance in curing rickets (in chickens) was the emission line of wavelength 2,894 angstroms. Considering the difficulties involved in evaluating the radiation intensity, his estimate that the line at 3,024 angstroms is about one fourth as effective as the maximum (at 2,967 angstroms) is in accord with more recent and more extensive research. In agreement with others, he found that the emission line at 3,132 angstroms has no appreciable curative value in healing rickets.

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[9] Hess, A. F., and Weinstock, Mildred: The Antirachitic Activity of Monochromatic and Regional Ultraviolet Radiations, Proc. Soc. Exper. Biol. & Med. **24**: 759 (May) 1927. Hess, A. F., and Anderson, W. T. Jr., id., J. A. M. A. **89**:1222 (Oct. 8) 1927.

[10] Sonne, C., and Rekling, E.: Behandlung experimenteller Rattenrachitis mit monochromatischen Ultravioletten Licht, Hospitalstid. **70**: 399 (April) 1927; Strahlentherapie **25**: 552, 1926.

[11] Maughan, G. H.: Ultraviolet Wavelengths Valuable in the Cure of Rickets, Am. J. Physiol. **87**: 381 (Dec.) 1928.

The most recent and most precise evaluation of the spectral antirachitic action of ultraviolet radiation is by Bunker and Harris.[12] The wavelengths of homogeneous radiation were isolated with a powerful quartz monochromator, and their intensities were evaluated radiometrically. More than 300 albino rats were used in these irradiation tests. Since complete healing (as judged by x-ray and line test) cannot be judged accurately, their criterion for judging antirachitic action was the degree of partial calcification known as the equivalent of one Steenbock unit of standard vitamin D. The principal emission lines of the mercury arc between 2,537 and 3,025 angstroms inclusive were demonstrated to have antirachitic properties, and the adjacent lines at 2,483 and 3,132 angstroms, respectively, were found inactive.

In chart 2 is shown the spectral antirachitic response for an equal energy spectrum, as deduced from the data published by Bunker and Harris.[12] The response curve is not necessarily smooth and free from indentations,[12a] although it is so depicted for the purpose of this discussion. In chart 2 also is given the spectral absorption of ergosterol.[2] From this it can be seen that if, as some suppose, the healing of rickets is associated in some manner with the activation of the sterols (ergosterol, cholesterol) present in the superficial layer of the skin, then, since photochemical action occurs only in the region of absorption, there should be a close parallelism between these two curves, as indicated. For the present this appears to be an interesting coincidence, the biologic significance of which awaits solution. For comparison there is shown in the chart the maximum lethal action (for an equal energy spectrum) on Bacillus coli.[13] This appears to lie in the region at 2,600 angstroms and is probably not true for bacteria in general. However, the fact that many kinds of bacteria survive the ultraviolet in sunlight indicates that wavelengths shorter than 2,900 angstroms may be expected to have the greatest lethal action.

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[12] Bunker, John W. M., and Harris, Robert S.: Precise Evaluation of Ultraviolet Therapy in Experimental Rickets, New England J. Med. **216**: 165 (Jan. 28) 1937.

[12a] Since this paper was written Arthur Knudson and Frank Beriford (J. Biol. Chem. **124**: 287 [June] 1938) have published a quantitative study of the effectiveness of ultraviolet radiation of various wave lengths in healing rickets, in which they found a maximum anti-rachitic response at 2804 angstroms and a minimum (indentation) at 2894 angstroms. The maximum at 2967 angstroms is about 20 per cent lower than illustrated in chart 2.

[13] Bachem, A.: Ultraviolet as a Bactericide, Arch. Phys. Therapy **16**: 733 (Dec.) 1935. Bachem, A., and Dushkin, M. A.: Proc. Exper. Biol. & Med. **30**: 700 (March) 1933.

The Spectral Erythemic Reaction.—In chart 2 is shown the average spectral erythemic reaction of the untanned skin to an equal energy spectrum,[14] based on the researches of Hausser and Vahle, of Luckiesh, Holladay and Taylor, of Coblentz, Stair and Hogue, and of Rüttenauer. Considering the fact that Hausser, Coblentz and Rüttenauer used wavelengths of homogeneous radiation isolated with a spectroradiometer as stimuli, whereas Luckiesh used wide bands of filtered radiation, there is good agreement among the various observers. One difficulty in estimating a threshold or minimum perceptible erythema (defined as a reddening of the skin that disappears in less than eighteen hours) is that for the longer wavelengths, for a slight overexposure, pigmentation is visible within twenty-four hours after exposure. Moreover, the minimum perceptible erythema is easily affected by the kind of diet (acid foods increase the erythemal reaction and pigmentation; alkali foods weaken erythema and pigmentation [15]) and also by external conditions. For example, wiping the irradiated skin with benzene or toluene causes the minimum perceptible erythema to disappear, and wiping with alcohol, twenty-four hours after irradiation, causes a reddening of some of the exposures that were invisible. Hence, before making an erythema test the parts that are to be irradiated should be washed with soap and water, thoroughly dried, and left untouched for twenty-four hours or more.

The spectral reaction differs from (and seems less complex than) the visual response in that there are two maximums, at 2,500 and 2,967 angstroms respectively. In contrast with the visual response, these maximums are not affected by the intensity or the spectral composition (the "color") of the source. This deduction follows from the close agreement between the observed and calculated erythematogenic efficiencies of various sources of heterogeneous ultraviolet radiation.[16]

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[14] Coblentz, W. W., and Stair, R.: Data on the Spectral Erythemic Reaction of the Untanned Human Skin to Ultraviolet Radiation, J. Research Nat. Bur. Stds. **12**: 13 (Jan.) 1934; **8**: 541 (April) 1932.

[15] Kittel, T., and Stahl, R.: Effect of Diet and Salves on the Erythemal Reaction and Pigmentation, Strahlentherapie **48**: 283 (Oct.) 1933.

By selecting a group of healthy persons of average skin pigmentation there seems to be no difficulty for a manufacturer to establish an erythema standard for judging the ultraviolet output of a lamp. This seems to follow from the fact that, from a knowledge of the ultraviolet spectral energy distribution of a lamp and the average spectral erythema response,[14] I have repeatedly verified, by calculation, the claims made by a manufacturer that his lamp produces an erythema in a certain time of exposure.

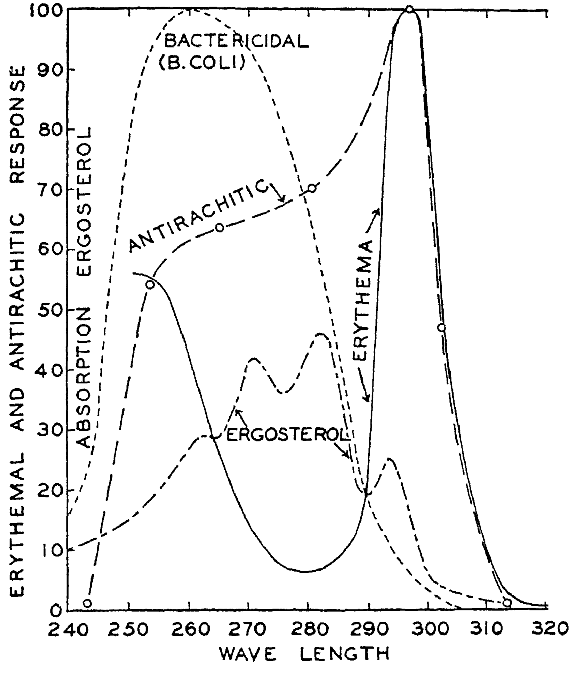

Chart 2.—The antirachitic response for an equal energy spectrum is shown, by circles connected by the broken line, as compared with the spectral absorption of ergosterol. The chart also shows the curve of lethal action for Bacillus coli and the average spectral erythemic reaction of the untanned skin to an equal energy spectrum.

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[16] Coblentz, W. W.; Stair, R., and Hogue, J. M.: Tests of a Balanced Thermocouple and Filter Radiometer as a Standard Ultraviolet Dosage Intensity Meter, J. Res. Nat. Bur. Stds. 8:759 (June) 1932.

As shown in chart 2, the wavelength limits of antirachitic and erythemal action are in close coincidence. Hence, since the time of exposure to ultraviolet radiation depends on skin tolerance as indicated by the erythemal response, it is apparent that, with the lamps now available in ultraviolet therapy, the time of exposure is limited by skin tolerance.

In response to a request for a simple means of determining whether a lamp emits ultraviolet radiation and in order to protect the public from imposition by purchasing a lamp that emits little or no ultraviolet radiation, the Council on Physical Therapy of the American Medical Association has adopted[17] and, until a more practicable and reliable procedure is proposed, will use the erythemal reaction as a basis for judging the effectiveness (the ultraviolet output) of a sun lamp. The Council does not prescribe the dosage, which is still highly empirical,[17a] and is left to the judgment of the physician.

SOURCES OF RADIATION FOR USE IN ULTRAVIOLET LIGHT THERAPY

From the coincidence of the spectral range of wavelengths of the erythemal and the antirachitic reaction it is evident that, with the sources of ultraviolet radiation now in general use (having a strong emission in the spectral region of 2,500 and 3,000 angstroms respectively), the dosage time of exposure that can be employed without causing a burn is determined by skin tolerance as measured by the erythemal reaction.

This is evident from a study of charts 3 and 4. Chart 3 depicts the distribution of energy in the ultraviolet spectrum of the carbon arc, cored electrodes impregnated with iron oxide (B carbon) and with the oxides of a variety of metals (C carbon) being used. The shaded area, which is the product of the relative spectral intensities and the spectral erythemic response (chart 2), shows the erythematogenic effectiveness in different parts of the spectrum (wavelengths; 310 millimicrons = 3,100 angstroms).

Similarly, in chart 4 is shown the ultraviolet spectral energy distribution (relative intensities) of the sun, of magnesium (Mg), and of various forms of the mercury vapor arc, in Corex glass and in fused quartz enclosures. Here the unshaded areas give the intensities of the individual emission lines and the dark areas give the relative effectiveness of each line in generating an erythema.

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[17] Council on Physical Therapy: Acceptance of Sunlamps, J. A. M. A. **102:** 42 (Jan. 6) 1934.

[17a] Luce-Clausen, Ethel M.: Clinical Aspects of Ultraviolet Therapy, J. A. M. A. **111:** 311 (July 23) 1938.

In the treatment of rickets by irradiation there is but little difference in the erythematogenic and antirachitic efficiencies of the various sources of ultraviolet radiation now in use. For example, on the basis of the results published by Bunker and Harris,[12] assuming that the spectral antirachitic response in human beings is the same as observed on experimentally induced rickets in rats (chart 2), the ratio of the calculated antirachitic efficiency of the ultraviolet radiation from various sources is as follows: "Therapeutic C" (poly-

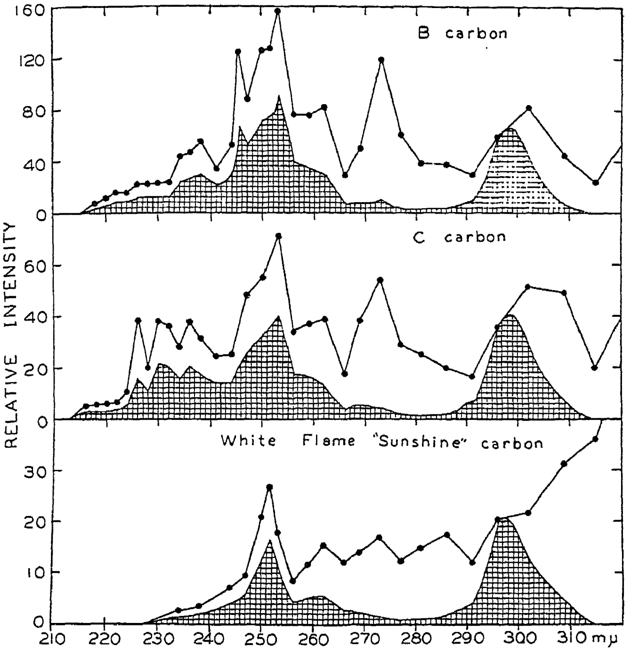

Chart 3.—Erythematogenic effectiveness in different parts of the spectrum. The distribution of energy is shown in the ultraviolet spectrum of the carbon arc, with cored electrodes (White Flame "Sunshine" carbon), electrodes impregnated with iron oxide (B carbon), and with the oxides of a number of metals (C carbon). The shaded areas represent the product of the relative spectral intensities and the spectral erythemic response.

metal) cored carbon arc (antirachitic ÷ erythemal) = 1.35; low voltage, high temperature mercury vapor arc in quartz (Uviarc) 1.14; high temperature, mercury vapor arc in Corex D glass (General Electric Company, S-1) 1.14; low vapor pressure, high voltage, low temperature (so-called cold quartz) mercury vapor arc in quartz 1.12 and midlatitude, midsummer, midday, sea level sunlight 0.95.

From this it can be seen that, of the various artificial sources now in use, only with the carbon arc impregnated with metals (Mg) having a strong emission in the spectral region of from 2,700 to 2,900 angstroms is it possible to deliver an appreciably (35 per cent) greater amount of antirachitic radiation than erythematogenic radiation, in the time fixed by skin tolerance as determined by the erythemal reaction.

This does not take into consideration the efficiency of antirachitic action as dependent on the before mentioned effect, possibly, of different wavelengths on calcium and phosphorus metabolism, and also on the deactivation effect of short wavelengths on vitamin D, which may militate against long exposures with sources having a strong emission of wavelengths shorter than about 2,800 angstroms, e. g., the so-called cold quartz lamp, in which over 95 per cent of the activating radiation is in the resonance emission line at 2,537 angstroms. On the other hand, such a source (cold quartz) permits an overexposure, by a factor of 5 or perhaps more, without causing a painful watery blister that results from a slight overexposure to sources of ultraviolet having a relatively strong emission of wavelengths 3,100 to 3,200 angstroms. From this it appears that, since the erythemal reaction is a measure of skin tolerance, it is indirectly a measure of the effectiveness of the sources of ultraviolet radiation now in use in healing rickets.

Incidentally it is of interest to note that the amount of ultraviolet radiation that can be applied practically to milk is influenced by the flavor that is developed; also that the antirachitic quality of the irradiated milk is proportional to the total ultraviolet energy imparted to the milk.[18] By using a carbon arc lamp with cored electrodes containing magnesium, which has a strong emission at 2,800 angstroms, more than three times the energy (of wavelengths 2,600-3,100 angstroms) could be imparted to the milk before a perceptible flavor developed, than with sources that are weak in radiation at 2,800 to 2,900 angstroms.

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[18] Weckel, K. G.; Jackson, H. C.; Haman, R., and Steenbock, Harry: Effect of Different Sources of Radiant Energy on the Flavor and Antirachitic Potency of Milk, Indust. & Engin. Chem. **28**: 653 (June) 1936.

Obviously in the healing of rickets, or in the production of vitamin D in milk, the time of exposure could be greatly prolonged if a source were available that emits radiation predominantly in the spectral band at 2,800 angstroms. However, there are no practicable filters available having a high transmission at 2,800 angstroms to isolate efficiently this spectral band of radiation in the commonest sources of ultraviolet radiation; and the spark discharge between the electrodes of magnesium, which has a strong emission in the region of 2,800 angstroms, is too noisy, too irregular in emission, and too inefficient for use as a source of ultraviolet having strong antirachitic action and a weak erythematogenic action.

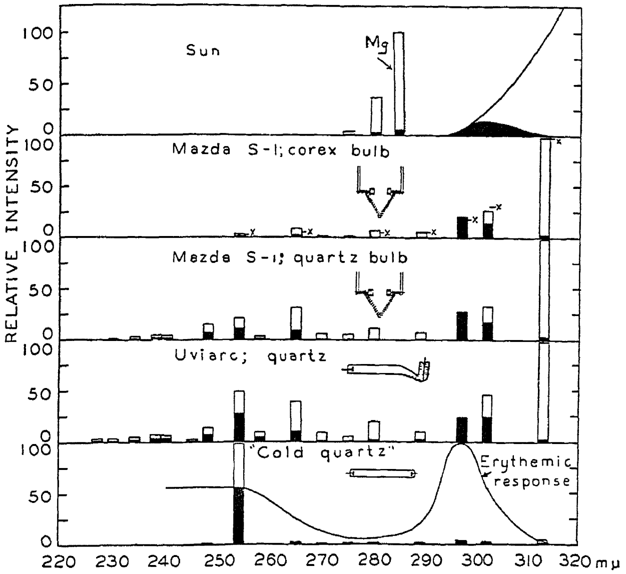

Chart 4.—Comparison of the ultraviolet spectral energy distribution of sunlight and the light from various forms of lamps. The unshaded areas represent intensities of individual emission lines; the dark areas represent the relative effectiveness of each line in producing an erythema.

In a recent discussion of this subject Seitz[19] quotes data published by Pincussen[20] on the emission spectrum of the low pressure discharge through magnesium vapor, in a special ultraviolet-transmitting glass bulb, in which there is strong emission at 2,796 angstroms and especially at 2,852 angstroms (see upper part of chart 4, Mg). The practical application of such a lamp remains to be determined. Hence in the meantime it will be more efficient to shorten the time of exposure to the ordinary sources of ultraviolet radiation in order to avoid production of a burn instead of using sources of filtered radiation emitting wavelengths only in the region of 2,800 angstroms, which have practically no erythematogenic action but which, because of their low intensity, require a long exposure for effective antirachitic action.

From the foregoing discussion it may be noted that since many sources of ultraviolet are weak in radiation of wavelengths shorter than 2,800 angstroms or have glass enclosures to intercept wavelengths shorter than 2,800 angstroms (in the case of the sun, the spectrum terminates at about 2,900 angstroms) the emission line at 2,967 angstroms is useful as a standard for evaluating the effectiveness of sources of radiation in terms of both the erythemal and the antirachitic reaction, particularly since the (dosage) time of exposure depends on skin tolerance, as indicated by the erythemal reaction.[16]

CONCLUSION

It is to be emphasized that the curative properties of a lamp are not necessarily measured by its power to generate an erythema; also that the dosage, whether erythemal or suberythemal, should be left to the discretion of the physician. However, in order that a lamp may qualify as a therapeutic agent it should emit sufficient ultraviolet to produce an erythema in a reasonable time of exposure (say fifteen minutes or shorter) if the physician desires to give an erythemal dose.

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19. Seitz, O.: Ultraviolet Radiators and Their Biologic Evaluation, Strahlentherapie **55**: 598, 1936.

20 Pincussen, L.: Handb. d. biolog. Arbeitsmeth. u. Abderhalden **5**: 13, 1933.

CHAPTER XXIX

CLINICAL ASPECTS OF ULTRAVIOLET THERAPY

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To write another article on the subject of ultraviolet therapy would seem, at first sight, to be quite inexcusable. There are few subjects on which more papers have been published, few subjects more ably covered in articles of review, and, one might add, no subject more capable of reducing to utter despair an author faced with the necessity of saying something new. My excuse for a repetition of this oft-told tale is the advisability of keeping before the medical profession subjects of medical interest and importance, and also the fact that with a growing literature new observations emerge which need to be linked with antecedent fact.

Since the last series of these articles appeared in 1932, the developments of the subject, as will be explained later, are mainly concerned with the extension of radiation therapy to diseases other than rickets and tetany, and to the somewhat conflicting observations as to possible dangers of the excessive use of sunbaths and general treatments with radiation. It is impossible, within the scope of a short article, to attempt anything comprehensive, so that the bibliography given makes no claim to be complete, and in general only the more recent literature has been quoted.

HISTORICAL

The classic paper of Palm[1] in 1890 is of increasing rather than of diminishing interest. At that time the method of geographic survey in the study of the etiology of disease was in vogue and was the method employed by Palm. The Collective Investigation Committee of the British Medical Association had already studied the etiology of various diseases, including

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From the Department of Biology, University of Rochester.

[1] Palm, T. A.: The Geographical Distribution and Aetiology of Rickets, Practitioner **14:** 270-79 and 321-42, 1890.

rickets, from the standpoint of their geographic distribution in the British Isles. This report was published in 1889.[2] Palm studied it and noted the following three points: (1) the frequency of rickets in large towns and thickly populated districts, and its rarity in rural areas, (2) the greater frequency of the disease in rural areas in the south than in the north of Great Britain, and (3) a direct ratio between the incidence of rickets in a community and the massing together of its population.

Palm was a traveled man, for after practicing medicine in England and Scotland he went to Japan. Here he lived for nine years and saw practice among all classes of the native population. The striking absence of rickets in Japan, where food and hygienic conditions were worse than in England, compared with its high incidence in the latter country, appeared to be the starting point of his investigation. He issued a questionnaire to medical missionaries in China, India, Tibet and Morocco. He gives, in extenso, their replies. These replies convinced Palm that the one factor common to all districts where rickets was unknown was sunlight; the converse was also clear to him from the British investigation already quoted.

He urges in his discussion a more careful study of the physiologic and therapeutic action of sunlight. He quotes the work of Darwin[3] in proof of the theory that light may strike an organism locally and its effect be transmitted. Darwin did a series of extremely interesting experiments on the effect of light on the bending of the coleoptile (called by Darwin the cotyledon) of Phalaris canariensis. These straight, cylindric coleoptiles normally bend toward the light. Darwin found that exposure of the tip of the coleoptile to light determined the bending of the lower part toward the light because, (*a*) when the tip of the coleoptile was either cut off or covered with a cap impermeable to light, bending did not occur, and (*b*) illumination of the lower part of the coleoptile, with the tip covered, produced no bending. Palm also recognized, from the work of Downes and Blunt, the bactericidal effect of "actinic rays" and urged in his final conclusion systematic and exact recording of "actinic rays" especially in sunless areas, the removal of rachitic children from crowded cities to sunny localities, the establishment of sanatoriums in such localities, the systematic use of sunbaths, and, finally, "the education of the public to the appreciation of sunshine as a means to health."

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[2] Geographical Distribution of Rickets, Acute and Subacute Rheumatism, Chorea, Cancer and Urinary Calculus in the British Isles, Brit. M. J. **1:** 113 (Jan. 19) 1889.

[3] Darwin, Charles: The Power of Movement in Plants, New York. D. Appleton & Co., 1888, p. 468.

It seems incredible that this paper should not have attracted attention at the time of its publication, but, like all sound clinical observations, it was destined to receive full merit as soon as the time was ripe.

In 1919 Huldschinsky [4] demonstrated by means of the x-rays the deposition of lime salts in the bones of rachitic children treated with exposures to the mercury vapor quartz lamp, and the truth of Palm's contentions became evident. To Huldschinsky is usually given the credit for this clinical discovery, although, as Park [5] points out, there is a possibility that Buchholtz made the same discovery many years earlier. It is interesting to note that Palm's suggestion, based on Darwin's experiments, that the effect of light is systemic and not local, was proved to be true when Hess [6] showed that by irradiating only one wrist of a rachitic patient the deposition of lime salts in the nonirradiated wrist could be observed.

This historical background has been emphasized in this way because it is, in essence, a clinical one. The clinical demonstration of the deposition of lime salts in bone following ultraviolet irradiation blazed a trail which led the experimentalist into many fields.

EXPERIMENTAL

For the purposes of the present paper a detailed account of the chemistry of vitamin D is not necessary. I [7] have reviewed the subject before and it has been reviewed by Bills [8] and others. For exhaustive articles of review the reader is referred to Park,[5] Korenchevsky,[9] Laurens,[10] Clouse[11] and Chick.[12] General articles dealing with the subject of ultraviolet radiation in relation to health have been writtetn by Clark[13] and Mayer.[14] Such articles as these afford ample evidence of the impossibility of saying anything on this subject that has not been said before, and said in the best possible way.

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[4] Huldschinsky, K.: Heilung von Rachitis durch künstliche Höhensonne, Deutsche med. Wchnschr. **45**: 712 (June 26) 1919.

[5] Park, E. A.: The Etiology of Rickets, Physiol. Rev. **3**: 106 (Jan.) 1923.

[6] Hess, A. F., and Unger, L. J.: The Cure of Infantile Rickets by Sunlight, J. A. M. A. **77**: 39 (July 2) 1921.

[7] Luce-Clausen, Ethel M.: The Therapeutic Value of Radiation, Medicine **8**: 419 (Dec.) 1929.

[8] Bills, C. E: Physiology of Sterols, Including Vitamin D, Physiol. Rev. **15**: 1 (Jan.) 1935; New Forms and Sources of Vitamin D, J. A. M. A. **108**: 13 (Jan. 2) 1937.

ULTRAVIOLET THERAPY AND RICKETS

Rickets is a disease caused primarily by lack of sunlight; it is a disease of sunless areas, of winter months; a disease caused by artificial conditions which exclude sunlight, such as the massing together of the population in tenement houses and, in the East, of living in purdah. The influence of ultraviolet radiation on rickets may be considered from (1) the physiologic and (2) the clinical point of view.

Physiologic.—The curative effect of ultraviolet radiation on rickets and its prophylactic use in the prevention of the disease are based on known physiologic facts. Diets constituted in such a way that the ratio of calcium to phosphorus is high, usually about 4:1, and also deficient in vitamin D, will produce severe rickets in rats; young rats, placed on such a diet, will survive the diet only from six to seven weeks, and at autopsy will show all the classic signs of severe rickets. A short daily exposure to ultraviolet rays will, in spite of the deficiencies in the diet, make these rats survive as long as twenty-five weeks. They may die, but with normal bones. The mechanism that makes an organism capable of supplying mineral constituents for bone formation, even in spite of a shortage of it in the diet, is set in motion by ultraviolet radiation. That this effect is a chemical one and not brought about by some integrating mechanism in the body such as the nervous system, is shown by the following: Hess and

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[9] Korenchevsky, V.: The Aetiology and Pathology of Rickets from an Experimental Point of View, M. Res. Council, Special Report series 71, 1922.

[10] Laurens, Henry: The Physiological Effects of Radiation, Physiol. Rev. **8**: 91 (Jan.) 1928; Physiological Effects of Radiant Energy, Monograph Series 62, New York, Chemical Catalog Company, Inc., 1933, p. 297.

[11] Clouse, Ruth Cowan: Vitamin D, J. A. M. A. **99**: 215 (July 16), 301 (July 23) 1932.

[12] Chick, Harriette: The Relation of Ultraviolet Light to Nutrition, Lancet **2**: 325 (Aug. 13) 377 (Aug. 20) 1932.

[13] Clark, Janet Howell: Ultraviolet Radiation in Relation to Health, Nutrition Abstr. & Rev. **3**: 13 (July) 1933.

[14] Mayer, Edgar: The Present Status of Light Therapy: Scientific and Practical Aspects, J. A. M. A. **98**: 221 (Jan. 16) 1932.

Weinstock[15] took some excised human skin and calf skin and irradiated it with a mercury vapor quartz lamp. Weighed amounts of this when fed to rats fed on a rickets producing diet proved to be completely protective, while similar amounts of nonirradiated skin were without effect. Light had conferred on the skin, although severed from its nerve and blood supply, antirachitic properties. In the same way, a rickets producing diet can be made protective by exposure to ultraviolet radiation.

The Rays Responsible for this Effect: In the solar spectrum the protective region is a very narrow band of radiation ranging from 296 to 310 millimicrons and furthermore these radiations must be present in sufficient intensity to produce their effect.

Hess and Anderson[16] working with a monochromatic illuminator, found antirachitic activity of wavelengths at 313 millimicrons to be very feeble, and they define this as the upper limit of the antirachitic field. It is obvious, therefore, that the rays responsible for the prevention and cure of rickets are very few, and, as these writers point out, during the winter months, when the shorter ultraviolet rays fail to reach the earth and the longer ones are less intense, "the width of the antirachitic zone of sunlight is only about 5 m μ." Furthermore, this band of radiations is easily filtered out by such substances as moisture, dust and smoke.

Artificial sources of light, such as the carbon arc or the mercury vapor quartz lamp, emit radiations of shorter wavelength than those found in the solar spectrum. Sonne and Rekling[17] showed that some of these—the region about 280 millimicrons—are markedly antirachitic; others around 248 and 240 millimicrons are less so.

Penetration of the Rays: Many writers have investigated the depth to which ultraviolet rays penetrate the skin. Most of them are agreed that the penetration is slight, roughly only 0.1 mm.[18] Measurements

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15. Hess, A. F., and Weinstock, Mildred: The Antirachitic Value of Irradiated Cholesterol and Phytosterol, J. Biol. Chem. **64:** 181 (May) 1925.

16. Hess, A. F., and Anderson, W. T.: The Antirachitic Activity of Monochromatic and Regional Ultraviolet Radiations, J. A. M. A. **89:** 1222 (Oct. 8) 1927.

17. Sonne, Carl, and Rekling, Eigil: Behandlung experimenteller Rattenrachitis mit monochromatischem ultravioletten Licht, Strahlentherapie **25:** 552, 1927.

18. Clark, Janet: The Physiological Action of Light, Physiol. Rev. **2:** 277 (April) 1922.

of radiation that is absorbed, transmitted and reflected by the skin are admittedly very difficult to make and give variable results. Skin is not homogeneous in an optical sense, and light incident on it is largely reflected and scattered. Anderson and Macht[19] point out that the depth of penetration depends on viability and that rays penetrate more deeply into living than into dead or moribund skin. These authors showed a penetration of 1.2 mm. through living tissue.

The Mode of Action of Ultraviolet Radiation: To understand how radiation brings about its effect in the cure of rickets one must have in mind two well known facts. First, that in the development of the disease there is the local phenomenon of failure or delay in calcification taking place at the epiphyses of bone, and, second, that there is the systemic one shown by a chemical change in the blood resulting in a diminution of the concentration of phosphate. Rickets, then, is something more than a disease localized in bone; it is regarded as a general metabolic disorder. Ultraviolet radiation raises the phosphate concentration in the blood and also promotes the deposition of lime salts in bone, through the agency of vitamin D which is synthesized in the skin. It is assumed that vitamin D, formed in the skin, is absorbed into the blood stream and carried to the bones. This theory is supported by the work of Hume, Lucas and Smith,[20] who showed that vitamin D could be absorbed through shaven areas of skin in rabbits, and more recently Lucas,[21] in a series of ingenious in vitro experiments, has shown that sufficient ultraviolet of suitable wavelength can penetrate the epidermis. It therefore might activate provitamin D contained in the subepidermal capillaries. The mechanism whereby lime salts are actually laid down in the epiphyses is not clearly understood. There is a vast and complicated literature on this subject but the modus operandi is still obscure. Robison, in a series of important papers on calcification in vitro, has demonstrated the presence in the epiphyses of bone and in other tissues of phosphatase, a bone enzyme. The part played by this enzyme and vitamin D in the absence of calcification when rickets is present, or in the production of calcification during its cure, is still uncertain. It would seem, however, that the concentration of phosphate in the blood is the factor that controls the deposition of lime salts, for, clinically speaking, it is unusual to detect healing in the bones by x-rays until a rise in blood phosphate has been obtained.

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[19] Anderson, W. T., and Macht, D. I.: The Penetration of Ultraviolet Rays into Live Animal Tissue, Am. J. Physiol. **86**: 320 (Sept.) 1928.

[20] Hume, E. M.; Lucas, N. S., and Smith, H. H.: On the Absorption of Vitamin D from the Skin, Biochem. J. **21**: 362, 1927.

[21] Lucas, N. S.: The Permeability of Human Epidermis to Ultraviolet Radiation, Biochem. J. **25**: 57, 1931.

Clinical.—Dorno [22] in 1919 published a book giving results of measurements of the radiations from the sun in Davos at different times of the year. He showed by means of graphs great variations in the intensity of the ultraviolet of sunshine throughout the day, and also the greatly lessened intensity of ultraviolet on a day in January as compared with a day in July. Hess [23] made a graph showing the total hours of sunshine in New York, Berlin, Paris, Stockholm and Greenwich. He found that New York had the same number of total hours of sunshine in February and March as was recorded in London during June, July and August, but that rickets was prevalent in New York during February and March but underwent healing in London during the summer. Although children get outdoors more frequently in summer than in winter, this indicates that the quality and intensity of the protective radiations are of more importance than their duration.

This seasonal variation in the intensity of the antirachitic rays of the sun is of the utmost importance when one considers sunlight as a therapeutic agent. Studies of the seasonal incidence of rickets have shown very clearly that the peak of cases occurs during the winter months when the ultraviolet of sunlight is of lowest intensity; the converse is also true—the fewest number of cases occur in the summer months, and summer is the time when spontaneous healing is observed. Hess and Lundagen [24] demonstrated a seasonal tide of phosphates in the blood of infants in New York City which was shown very clearly to be a winter ebb and a summer flow. This occurred in spite of the fact that the infants were exposed to sunlight every fine day. In most of the infants some degree of rickets developed during the winter months in spite of excellent hygienic conditions. Hess regards a winter reduction of blood phosphate to be usual for infants living in the temperate zone.

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[22] Dorno, C.: Physik der Sonnen und Himmelsstrahlung Braunschweig, 1919.

[23] Hess, A. F.: The Ultraviolet Rays of the Sun, J. A. M. A. **84:** 1033 (April 4) 1925.

[24] Hess, A. F., and Lundagen, M. A.: A Seasonal Tide of Blood Phosphate in Infants, J. A. M. A. **79:** 2210 (Dec. 30) 1922.

The treatment of rickets with ultraviolet radiation is much to be recommended, since ultraviolet radiation is a potent and reliable specific in this disease. The radiations may be either those of sunlight or those obtained from artificial sources.

HELIOTHERAPY

The difficulty of using heliotherapy in the climate of the northern part of the United States during the winter is obvious from the preceding paragraph; the specific radiations are not available in sufficient intensity at a time when they are most needed, and, furthermore, the cold makes it difficult to expose much of the body surface to the direct action of the rays. Cold makes it also difficult to expose infants, however warmly clad, for a sufficient length of time. Hess[25] (p. 423) found the curative use of heliotherapy unsatisfactory during the entire months of January and February and the early part of March in New York City. It is clear from this that, during the winter, treatment should be given from artificial sources. The technic of using heliotherapy for infants has been described in practical detail by Hess[25] (p. 424) and is here quoted:

> The technic of carrying out heliotherapy is as follows: At first the legs are exposed, the feet being protected by socks in the early spring and autumn. The first exposure should be for ten minutes and is to be regarded as a preliminary test to gage the susceptibility of the individual. On each successive morning, the treatment may be prolonged five minutes until the infant is being exposed for one-half hour daily. On alternate days, the arms are treated in the same way—at the beginning for ten minutes and increasing the period as with the legs. As soon as weather permits, the trunk is exposed, the baby being clad merely in a diaper with the hands and feet covered. The head should always be protected with a light cap; the eyes may be shielded. Mothers and nurses are often greatly concerned lest the sunlight prove harmful to the eyes, but in my experience neither conjunctivitis nor any other injury to the eyes has resulted. As it is well known, blondes are in general more sensitive to ultraviolet rays than brunettes, but there are exceptions to this rule, so that care should always be exercised at the beginning of treatment. Every year I see one or more infants who have developed painful blisters or bullae on the

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[25] Hess, A. F.: Rickets, Osteomalacia, and Tetany, Philadelphia, Lea & Febiger, 1929.

face, as the result of exposure to the sun in the early spring. This burning can be prevented by applying a thin coating of oil or vaseline to the face, previous to placing the babies in the sunlight. It is not necessary to extend the exposure beyond one hour a day; in fact, in the warm weather, longer periods are contraindicated. In the summer, it is well to carry out heliotherapy during the early morning or late afternoon hours and not between 11 and 3 o'clock, when the heat is greatest. Following treatment, the infant should not become pale, nor should its rectal temperature rise. The results of heliotherapy in rickets are remarkable—the epiphyses rapidly calcify, the inorganic phosphorus in the blood increases, and there is marked improvement in the nutrition of the skin and the muscles. Benefit can be noted often in a fortnight.

IRRADIATION FROM ARTIFICIAL SOURCES

In using artificial sources of radiation it must of course be remembered that radiations are present of far shorter wavelengths than those emitted by the solar spectrum, and also that the antirachitic solar radiations, in these lamps, form a much higher percentage of the total rays. A rough estimate of the antirachitic radiations in sunlight in midsummer is 0.15 per cent of the total energy, while many artificial sources emit these rays in amounts as high as 20 per cent. The lamps most commonly used are the air-cooled mercury vapor and the carbon arc. The former emits far more of the short wavelength radiation than sunlight; the latter approximates more nearly in distribution to the radiations of sunlight but still transmits a much higher percentage of these rays. Dosage with these lamps is still highly empirical, but in general the dosage should induce a slight, but avoid a marked, erythema. Hess[25] (p. 425) recommends the following method:

> For the average case, the child is irradiated every other day for a total period of four weeks. In a severe case, the time may have to be extended to eight or ten weeks. If treatment is begun after January, it will not be necessary to repeat the course of treatment during the winter, but if it is begun in the autumn, a second series of irradiations should be given before March. The lamp is placed at a distance of 3 feet from the body and exposures of the front and back are carried out alternately. The exposures are at first for two minutes and are increased by one minute at every session, until a total of thirty minutes be reached. These rules can serve merely as a general guide and must be modified according to the individual case. Special care must be observed in the treatment of blondes and of infants which are markedly atrophic.

The effect on the rachitic process brought about by irradiation with the mercury vapor lamp is remarkable. Within ten to fourteen days radiographs show new calcification at the epiphyses as well as calcification of the carpal centers. However, neither irradiation from the sun nor from any artificial source confers a prolonged protection against rickets. Time and again, I have observed a second attack develop in spite of a thorough course of irradiation during the previous winter.

ULTRAVIOLET THERAPY AND TETANY

Idiopathic tetany of infancy, the symptoms of which are well known, has an intimate relationship to rickets. The age incidence of the disease is the same; it occurs very frequently in Negroes and premature infants; its seasonal incidence is even more marked than in rickets; the peak of the cases occur in early spring and practically no cases are seen in summer; nearly all infants with tetany, except those seen in the first two months of life, show at the same time some degree of rickets. The converse of this last point, however, is not true; rickets commonly occurs without manifestations of tetany, and there seems to be no parallelism between the severity of rickets and the tendency to develop tetany.

The blood chemistry in rickets shows a diminution in the concentration of phosphates in the serum. In tetany the phosphate concentration of serum remains unchanged and may even rise, but the level of calcium in the serum drops from the normal of 9 to 11 mg. per hundred cubic centimeters to 7 or 8 mg. in latent cases, and in cases presenting manifest symptoms it may be lower than this.

Ultraviolet radiation, cod liver oil and activated ergosterol are just as specific and effective in the cure of tetany as they are in rickets. It has been found, however, in certain cases, that symptoms of tetany may be intensified at the outset of treatment. This is thought to be due to a sudden rise of blood phosphate caused by the treatment, taking place before the level of calcium in the serum has risen. In this connection Hess and Lundagen,[24] in discussing the seasonal tide of phosphates in the blood of infants, incline to the view that the increase in the concentration of blood phosphate which occurs in April and May may be a contributing factor to the development of tetany in the late spring.

Ultraviolet therapy in tetany is of the utmost value. Hess recommends its use as a prophylactic against tetany for all premature infants, as well as its use as a specific cure once the disease has occurred. Treatment is carried out in the same way as that outlined for rickets. It may be advisable to give doses of feeble intensity at first until the level of calcium in the serum rises, and this should be combined with calcium therapy. Hoag[26] in a detailed study on the use of ultraviolet treatment in this disease found that it took, on an average, fourteen days' treatment to bring the calcium back to a normal level; he used a mercury vapor quartz lamp at a distance of 50 cm. and estimated that a total exposure of fifty minutes was needed to bring about an increase of 1 mg. of calcium per hundred cubic centimeters of serum. Full details of the treatment are given in this paper, with case histories.

ULTRAVIOLET THERAPY AND HEALING OF BONE FRACTURES

Since ultraviolet radiation is known to synthesize a powerful calcifying agent, vitamin D, in the skin, it is logical to suppose that such a substance, if absorbed into the blood stream, might promote rapid healing of bone in cases of fracture. A careful study of this possibility has recently been made by Sweeney and Laurens.[27] These authors studied the effect of carbon arc radiation on fractures produced experimentally in the fibulas of twenty-five normal dogs and eighty normal rats. The radiations from the lamp, operated at between 24 and 27 amperes, were carefully measured with regard to (*a*) total energy emitted and (*b*) the distribution of visible, infra-red and ultraviolet radiations from two types of carbon; the "sunshine" and the C carbons of the National Carbon Company, used by these authors. In the case of dogs, the fibula on one side was fractured and allowed to heal; the second fibula was then fractured and allowed to heal under daily exposures to radiation. A comparison was then made of the total length of the healing process with and without irradiation in the same animal. In the case of rats, uniform lesions were made by excising 1 mm.

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[26] Hoag, L. A.: The Treatment of Infantile Tetany with Ultraviolet Radiation, Am. J. Dis. Child. **26:** 186 (Aug.) 1923.

[27] Sweeney, H. M., and Laurens, Henry: The Effect of Carbon Arc Radiation on Healing of Bone, Arch. Surg. **31:** 395 (Sept.) 1935.

out of the middle of the shaft of the fibula. A comparison was then made of the percentage number of fractures which healed in a given time with and without radiation. The results may be summarized as follows: In dogs the average healing time was 3.7 days shorter in the irradiated group, but determinations of the calcium and phosphorus in serum showed that no connection existed between the calcium and phosphorus product and the rapidity of healing. In rats, 73.9 per cent of a group given radiations from "Sunshine" carbons, 26.9 per cent of a group given radiations from C carbons and 41.3 per cent of a group of nonirradiated control animals, healed in a given time. Laurens attributes the retardation of healing in the group of rats irradiated from the C carbons not to a higher percentage of ultraviolet and infra-red rays emitted by these carbons but to the proportion of these rays in the spectrum. This is an interesting point, which merits further study. In these experiments the shortening of the healing period with radiation that the authors found in dogs is probably too small to be of clinical significance, but such studies are most valuable because so carefully controlled. This study shows that the calcium and phosphorus product in blood serum is of little prognostic value in experimental conditions such as those described, but, as the authors point out, different results might be obtained in certain cases of fracture due to abnormal bone metabolism. It is also clear from this work that radiation is not a strong therapeutic agent in promoting the healing of bone fractures.

ULTRAVIOLET THERAPY AND TUBERCULOSIS

Ultraviolet radiations, usually from sunlight and the carbon arc lamp, continue to be used extensively in the treatment of tuberculosis. Expert opinion as to its value, however, is not in agreement. Coulter and Carter [28] have recently published an unbiased, carefully controlled clinical study of the treatment of pulmonary tuberculosis with ultraviolet radiation. The authors state that the object of this study was to obtain "facts and not opinions." They planned the work in such a way that an objective examination of bloods was made without any knowledge as to whether or not the patient had been irradiated. The inclusion of a series of non-irradiated patients as controls makes the study more valuable. They conclude that exposures sufficient to produce a mild erythema and subsequent pigmentation produced "no beneficial effects in cases of active pulmonary tuberculosis or in cases following thoracoplasty." In their series of cases ultraviolet irradiation had no uniform effect on the red blood cell count, the hemoglobin value or the white blood cell picture.

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[28] Coulter, J. S., and Carter, H. A.: The Treatment of Pulmonary Tuberculosis by Ultraviolet Radiation, J. A. M. A. **105**: 171 (July 20) 1935.

Banyai,[29] on the other hand, on the basis of treating 115 patients by Rollier's method, reports satisfactory therapeutic results "that justify the further application of this treatment in pulmonary tuberculosis and in tuberculous pleurisy." This author quotes an analysis of the literature made by Coulter and Carter which shows that, since 1902, out of seventy competent observers forty-six had favorable and twenty-four unfavorable results with the use of ultraviolet radiation in pulmonary tuberculosis.

Mayer [14] does not recommend light therapy for pulmonary tuberculosis and says that reliance on it as an important aid in this disease is not to be encouraged. He regards radiation as an aid in certain cases of extrapulmonary cases, such as "pretubercular" conditions in childhood, tuberculous lymph nodes (including hilus) pleura, bones and joints, peritoneum and intestine. His point of view that "in tuberculosis, light of any form by itself is not curative but comprises only one of the important adjuvants in treatment" must, at the present stage of our knowledge, be regarded as the safe and the scientific position.

ULTRAVIOLET THERAPY AND SKIN DISEASES

Erysipelas.—Several articles have appeared recently on the treatment of erysipelas with ultraviolet radiation. Davidson [30] reports on the treatment of fifty-one cases, giving exposures from an air-cooled quartz lamp of one and a half the erythema dose for normal skins. The radiation is directed locally onto the inflamed area with a margin beyond of 3.8 cm. of healthy skin when possible; the rest is protected from light. The exposure is five minutes with the arc at a fixed distance of 30 cm.; usually one exposure is sufficient. Davidson claims better results with this than with other methods in use; one should consider also Desmaison,[31] who prefers the Hanau Lamp and gives from three to six treatments, and Nightingale and Starr.[32]

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[29] Banyai, A. L.: Carbon Arc Treatment in Pulmonary and Pleuritic Tuberculosis, Arch. Phys. Therapy **18:** 205 (April) 1937.

[30] Davidson, J. M.: Phototherapy in Erysipelas, Brit. M. J. **1:** 929 (May 21) 1932.

It would seem to be advisable, before accepting irradiation as the treatment of choice in this disease, to study a group of cases in which only alternate cases are treated with radiation and the others with some other form of therapy. Results would then give undoubted evidence as to the relative value of the two forms of treatment. Also, since in erysipelas there is such well known seasonal variation in the virulence of the disease, studies should extend over a period of years, and the results observed in different years should be compared.

Psoriasis.—Goeckerman[33] recommends a combination of crude coal tar ointment and ultraviolet radiation as the treatment of choice in psoriasis. Details of the treatment are given in his paper.

Leprosy.—During the past four years an attempt has been made in the Netherlands East Indies to treat leprosy with ultraviolet radiation. This method of treatment, used at first with success, is still in the experimental stage.[34]

For a critical article which examines the claims made for ultraviolet therapy in dermatology, the reader is referred to MacKee.[35] This article contains a specialist's description of many skin diseases and states that the general belief that ultraviolet radiation is good for the complexion is "not in agreement with the consensus of dermatologic opinion." The author discusses the "farmer's or sailor's skin," thought to be due to excessive exposure to light, and which appears wrinkled and dry and in which keratoses of the senile type are apt to develop.

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31. Desmaison, R. F.: Treatment of Erysipelas by Ultraviolet Rays, Crón. méd., Lima **53**: 249 (July) 1936.

32. Nightingale, L. M., and Starr, Saul: The Treatment of Erysipelas in Children, J. A. M. A. **102**: 761 (March 10) 1934.

33. Goeckerman, W. H.: Tar and Ultraviolet Radiation in Treatment of Psoriasis, Brit. J. Phys. Med. **7**: 215 (March) 1933.

34. Ultraviolet Rays in the Treatment of Leprosy, Netherlands letter, J. A. M. A. **105**: 61 (July 6) 1935.

35 MacKee, G. M.: Ultraviolet Therapy in Dermatology, J. A. M. A **98**: 1553 (April 30) 1933.

GENERAL COMMENTS ON THE USE OF ULTRAVIOLET RADIATION IN THE TREATMENT OF SKIN DISEASES

From experimental knowledge it would seem that the various effects of radiation on the skin must be brought about mainly in three ways: 1. A direct bactericidal action of the rays on organisms in the skin which lie within the depth to which the rays penetrate. This, as explained elsewhere, is a superficial layer, so it seems clear that organisms, if they are to be killed by the direct action of radiation, must lie superficially. The well known beneficial effects of the Finsen treatment for lupus are probably explained as due to a direct bactericidal effect of the radiations used. It is also probable that the good results recorded in the use of ultraviolet radiation in the treatment of erysipelas are due to the same cause. Ultraviolet radiation may kill bacteria outright or attenuate their virulence if the organisms are given a sublethal exposure. It is thought that the latter effect may account for seasonal variations in the virulence of certain diseases. 2. An effect due to hyperemia. Ultraviolet radiation produces an initial hyperemia of the skin, which may be of value in certain diseases of obscure etiology and when no causative organism has been isolated, such as psoriasis. 3. A possible effect, at present not well understood, of substances synthesized in the skin under the action of radiation. With the exception of the production of tan and the synthesis of vitamin D, the photodynamic effects of radiation on the skin are not well understood. That certain skin diseases occur as a result of photosensitization seems well established, but how this condition is brought about is not known. The reader is referred to a recent article by Mathews[36] giving a complete account of this subject, with an exhaustive bibliography.

Peacock[37] has recently observed a fluorescence in the horny layer of the skin under the action of ultraviolet radiation. He points out that this is a protective mechanism against the destructive action of the rays, comparable to the fluorescence which occurs in the cornea and lens. Proliferation of the horny layer takes

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36. Mathews, F. P.: Photosensitization and the Photodynamic Diseases of Man and the Lower Animals, Arch. Path. **23**: 399 (March) 1937.

37. Peacock, P. R.: Skin Reaction to Ultraviolet Radiation, Brit. J. Phys. Med. **8**: 173 (March) 1934.

place at the same time as the formation of pigment; the latter protects the dermis from the action of radiation.

In general one may say that the reaction of the skin to ultraviolet radiation is designed mainly to protect the body from injurious effects. This fits in with the observations of dermatologists that the "weather-beaten" skin is not necessarily a healthy skin but one with a tendency to senile degeneration and the formation of keratoses. It is also probably extremely significant that skin cancers are rarely, if ever, seen in Negroes. The question should therefore be raised, in view of the general popularity of sun bathing and of irradiation from artificial sources, whether the overuse of radiation could predispose to cancer.

THE EXPERIMENTAL PRODUCTION OF SKIN TUMORS WITH ULTRAVIOLET RADIATION

Malignant tumors have been produced in rats and mice as a result of excessive exposure to ultraviolet radiation given either as sunlight or from artificial sources. Findlay[38] in 1928 exposed albino rats to radiation from an ultraviolet lamp at a distance of 18 inches (46 cm.) for one minute three times a week; in one out of a group of six animals a papilloma of the right ear developed and it died of senility after two years' treatment. Similar results were obtained with mice.[39] The exposures given by Findlay compared with those of later writers were of very short duration. Beard, Boggess and von Haam,[40] applying the technic of Roffo—i. e., twenty hours of ultraviolet radiation daily for one year—were able to produce sarcomas and carcinomas on the eyes, ears and heads in twelve out of a series of thirty adult rats. The lesions in these animals began to appear after two months of this treatment. Roffo[41] found in his early studies that a rat tumor may contain more than twice as much cholesterol as that found in the host. He exposed rats to sunlight, and to radiations from a Hanau lamp for long periods; he analyzed skin for cholesterol and found high values in irradiated as compared with nonirradiated skin. He obtained neoplasms in a high percentage of his irradiated animals. He suggests that irradiation, by causing an accumulation of cholesterol in the skin, may prepare the soil for subsequent malignant growth. As a result of this work, a committee was appointed by the Academy of Medicine in Paris to verify the observations of Roffo. The committee confirmed the observations and in view of the potential dangers warned the general public against the abuse of sun baths. Beard and his associates,[40] however, point out that such a warning may be unnecessary on the ground that (*a*) the physiologic response of the rat to ultraviolet radiation is greater than that of man and (*b*) the massive exposures necessary, even for the rat, to produce lesions, leave a wide margin of safety for man. It is also possible that the increased concentration of skin cholesterol produced by Roffo could be produced by other means, such as infections or irritants, and need not necessarily be regarded as a specific effect of radiation. Murray [42] regards the production of tumors by ultraviolet radiation as merely the effect of an irritant and comparable to the effect of coal tar, x-rays, radium and animal parasites. If this is so, it would seem that, since such extraordinarily long and continuous exposures are required to produce cancer in an animal as sensitive as the rat, man is in no danger. But if it should be proved that the effect of ultraviolet radiation is more specific, if for example the carcinogenic hydrocarbons could be synthesized in the skin by exposure to radiation, then there would be more cause for alarm. Cook,[42] in a discussion of carcinogenic substances, mentions this as a possibility but hazards it only as a speculation to stimulate further research.

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[38] Findlay, G. M.: Cutaneous Papillomata in the Rat Following Exposure to Ultraviolet Light, Lancet **1**: 1229 (June 7) 1930.

[39] Findlay, G. M.: Ultraviolet Light and Skin Cancer, Lancet **2**: 1070 (Nov. 24) 1928.

[40] Beard, H. H.; Boggess, T. S., and von Haam, Emmerich: Experimental Production of Malignant Tumors in the Albino Rat by Means of Ultraviolet Rays, Am. J. Cancer **27**: 257 (June) 1936.

[41] Roffo, A. H.: Heliotropism of Cholesterol in Relation to Skin Cancer, Am. J. Cancer **17**: 42 (Jan.) 1933.

At the Third International Congress for Light held at Weisbaden in 1936 [42a] several papers on the subject of skin cancer caused by ultraviolet radiation appeared followed by a discussion. Fundling, Henriques and Rekling (p. 166) described experiments with mice. Group A exposed to ultraviolet radiation for 12 months; exposures 10 minutes every other day. Results—by macroscopic examination. Forty-four per cent of the animals showed cancerous lesions, but microscopic examination of tissue raised the total to 95 per cent of the animals. Group B exposed for 4 months; exposures 10 minutes every second day. Results—cancerous lesions in 12 per cent of the cases. Shorter exposures than these gave negative or doubtful results. Wavelengths shorter than 280 mμ were found to be responsible for the effect.

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[42] Murray, M. A.: Discussion on Experimental Production of Tumours, Proc. Roy. Soc. London, series B **113:** 272, 1933.

[42a] Dritter Internat. Congress Für Lichtforschung, Weisbaden, 1936. Pub. by L. Schumacher, Berlin, N4, Chausseestrasse 42.

Holtz (p. 169) working with gray and white rats, giving exposures varying from 5 minutes to as long as 6 hours daily found that tumors began to develop in about 27-36 weeks after irradiation, and that the occurrence of these tumors, provided the time of exposure exceeded the threshold dose, was *independent of the daily time of exposure.* He states that the fact that tumors can be produced by short exposure to ultraviolet radiation makes it necessary to consider the possible dangers of sun-bathing for man, especially in high altitudes, and also emphasizes the possible dangers of the excessive use of quartz lamps. He thinks that the use of excessive radiation may cause a predisposition to the later development of malignant tumors. In a discussion (p. 171) Dr. Henriques gave as his personal opinion that great caution must be used in drawing conclusions with regard to man based on experimental work with animals.

In general, one might conclude that experiments such as these indicate a possible but not a very probable danger for man. However, the experiments of Holtz, which indicate that time of exposure to radiation may not be as important as the threshold dose, needed, in individuals, to produce tumors, should be noted. They raise the question of special susceptibility in man, and indicate the possible danger that the induction period to radiation might be shorter in such individuals. It also must be remembered that many lamps emit the radiations found by these authors to be carcinogenic. It is obvious that treatments given should remain within the physiologic limit of tolerance.

ULTRAVIOLET THERAPY AND ORAL DISEASE

A recent report[43] made at the request of the Council on Physical Therapy by the Council on Dental Therapeutics states that the claims for the efficacy of ultraviolet radiation in the treatment of oral disease, such as pain from various causes following the extraction of teeth, have not been substantiated.

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[43] Ultraviolet Therapy in Oral Diseases, editorial, J. A. M. A. **102**: 135 (Jan. 13) 1934.

GENERAL CONCLUSIONS AS TO THE VALUE OF ULTRAVIOLET THERAPY

The value of ultraviolet radiation in the prevention and cure of rickets and tetany is an accepted fact and has been proved indisputably to be both safe and specific if given under the conditions outlined.

In the treatment of fractures of bone, experimental evidence points to radiation as being of little if of any value.

In the treatment of tuberculosis, no claims for the specificity of ultraviolet radiation have yet been substantiated, though many authors still regard irradiation as a useful aid to other forms of treatment.

In the treatment of skin diseases of bacterial origin ultraviolet radiation may be of value, provided the organisms lie within the range to which the rays penetrate and are killed or attenuated by doses safe for the host. In other skin diseases, such as psoriasis, beneficial results might be due to the effect of radiation in producing hyperemia.

Skin tumors have been produced in rats and mice with prolonged exposure to ultraviolet radiation, but the exposures needed are so far outside the range in general use by man, either in sun bathing or in the use of rays from artificial sources, that a warning of danger seems unnecessary. A caution, however, to avoid the abuse of radiation therapy, since its effects on the skin are imperfectly understood, is, at this stage of our knowledge, completely justified. More research is undoubtedly needed on the question of the photodynamic effect of radiation on the skin with special reference to the possible synthesis, in the skin, of the carcinogenic hydrocarbons.

CHAPTER XXX

VITAMIN E

H. A. MATTILL, PH.D.
IOWA CITY

Some fifteen years ago several investigators in animal nutrition began to suspect that for normal reproduction the rat required a dietary constituent not yet recognized or included within the generally accepted group of accessories. At first there was some hesitation in accepting yet another member into the already large family of vitamins, but convincing evidence from various laboratories, especially from that of Evans and his co-workers, soon established the fact that when reared on diets otherwise complete, but not containing this new fat-soluble factor, rats did not have offspring, although they appeared to be quite normal in other respects. Male animals became infertile through degeneration of the germinal epithelium and the damage was irreparable. Females failed to carry their young to term; the embryos died and were absorbed, but the female reproductive mechanism as such was not damaged, since adequate dosages of the missing factor restored fertility. Still other experiments showed that diets in which this factor, although originally present, had been destroyed by oxidation were likewise inadequate for reproduction.

Thus vitamin E came into its own. In the five years that have elapsed since the publication of the previous symposium on vitamins in THE JOURNAL, our knowledge of vitamin E has been advanced considerably. It has been shown that at least three substances possess the effect of vitamin E. Their chemical nature is known, and the most potent of them, now called alpha tocopherol, has been prepared synthetically and found to be the physical, chemical and physiological equivalent of the naturally occurring substance. The need for vitamin E in other species, especially in domestic animals, its rôle in reproduction and possibly in other physiologic processes, its possible therapeutic value in preventing habitual abortion—these and other aspects

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CHEMISTRY

A suitable starting point for a chronological presentation of the chemical status of vitamin E is its association with antioxidants in nature. The antioxidants here concerned are substances which delay or prevent the auto-oxidation of fats and the rancidity resulting therefrom. The probable presence of these oxidation-inhibiting agents in certain vegetable oils, notably in wheat germ oil, which is rich in vitamin E, was deduced from the protection which this oil afforded against rancidity in certain experimental rations which were particularly susceptible to this form of oxidation. An actual association of antioxidant and vitamin E was first demonstrated in lettuce oil[2] and later in tomato and in wheat germ oil;[3] subsequently other vegetable sources were also found to contain both.

The ready susceptibility of vitamin E to oxidative destruction under favorable conditions is the basis of the common practice to include cod liver oil, butter fat or lard, sometimes in incipient rancidity, in experimental rations designed to be deficient in E; there are many illustrations[4] of the disappearance of vitamin E in the presence of these fats. Treatment of the food with ferric chloride[5] has been employed for the same purpose, the explanation being that oxidation destroyed vitamin E or the protecting antioxidants, or both.

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[1] Evans, H. M.: Vitamin E, J. A. M. A. **99:** 469 (Aug. 6) 1932.

[2] Olcott, H. S., and Mattill, H. A.: The Unsaponifiable Lipids of Lettuce: III. Antioxidant, J. Biol. Chem. **93:** 65 (Sept.) 1931.

[3] Bradway, Elizabeth M., and Mattill, H. A.: The Association of Fat-Soluble Vitamins and Antioxidants in Some Plant Tissues, J. Am. Chem. Soc. **56:** 2405 (Nov.) 1934.

[4] Macomber, Donald: Studies of Reproduction of the Rat: III. Vitamin E Neutralized When Mixed with Lard, New England J. Med. **209:** 1235 (Dec. 14) 1933. Ringsted, Axel: Histological Investigations on the Causes of Sterility in Albino Rats Kept on a Normal Butter Diet, Acta path. et microbiol. Scandinav. **11:** 197 (No. 2) 1934; through Biol. Abstr. **9:** 11407 (No. 6) 1935. Lassen, H. K.: The Influence of Butter Fat and Peanut Oil on the Growth and Fertility of Rats, Acta path. et microbiol. Scandinav. **11:** 183 (No. 2) 1934.

[5] This was first suggested by Waddell and Steenbock and has been employed in some of the experiments to be reviewed here. According to M. W. Taylor and V. E. Nelson (Some Observations on Ferric Chloride Addition to the Diet, Proc. Soc. Exper. Biol. & Med. **27:** 764 [May] 1930), both the nature of the diet and the manner of applying the ferric chloride determine the result. The procedure destroys vitamin E, but the partial destruction of vitamin A, or of other unknown essentials, and the possible toxicity of the salt itself are all variables that often make the interpretation of results difficult. See also Waddell, James, and Steenbock, Harry: Vitamin E in Iron Treated Dry Rations, J. Nutrition **4:** 79 (May) 1931.

In order to examine the relations between vitamin E and antioxidants more carefully, Olcott and Mattill[6] prepared a concentrate of vitamin E from wheat germ oil, which was found to have antioxygenic action as well as the biologic activity of vitamin E. This concentrate proved to be similar to the one first made by Evans and Burr some years ago and described in their monumental memoir on vitamin E. It differed in one respect from theirs: acetylation did not destroy the biologic activity although antioxygenic action was abolished. Hydrogenation was again found not to destroy the biologic activity (nor did it abolish the antioxygenic action) but the hydrogenation was incomplete even under very drastic treatment, as had been found by others.[7] The destructive action of chlorine and bromine was reversible; boiling with zinc and hydrochloric acid in methanol restored biologic activity. Mild oxidation with silver nitrate seemed to reduce but not destroy vitamin E activity, but treatment with more vigorous oxidants such as potassium permanganate, ozone and perbenzoic acid did so.[8]

Of greatest chemical interest was the biologic activity of acetylated or benzoylated concentrates; the formation of such derivatives from vitamin E would signify the presence of one or more hydroxyl groups. The antioxygenic action, which in these substances probably depends on a hydroxyl group,[9] was destroyed by such esterification; biologic activity was not; hence, if vitamin E possessed a hydroxyl group, either it was resistant to esterification or the ester was physiologically utilizable. By an interesting series of reactions Olcott[10] demonstrated that vitamin E does contain a hydroxyl group which is easily esterified and whose esters with acetic or benzoic acid are utilizable (hydrolyzable) by the animal body while a compound formed with phenyl isocyanate, for example, is not.

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[6] Olcott, H. S., and Mattill, H. A.: Vitamin E.: I. Some Chemical and Physiological Properties, J. Biol. Chem. **104:** 423 (Feb.) 1934.

[7] Bowden, F. P., and Moore, Thomas: Absorption Spectrum of the Vitamin E Fraction of Wheat-Germ Oil, Nature **132:** 204 (Aug. 5) 1933.

[8] Olcott, H. S.: Vitamin E: II. Stability of Concentrates Toward Oxidizing and Reducing Agents, J. Biol. Chem. **107:** 471 (Nov.) 1934.

[9] Olcott, H. S., and Mattill, H. A.: Antioxidants and the Oxidation of Fats: I. Inhibitols, J. Am. Chem. Soc. **58:** 1627 (Sept.) 1936.

[10] Olcott, H. S.: Vitamin E: III. Evidence for the Presence of a Hydroxyl, Group; The Biological Utilization of Esters; Absorption Spectrum, J. Biol. Chem. **110:** 695 (Aug.) 1935.

The presence of a hydroxyl group that could be acetylated was also demonstrated by Drummond and his co-workers[11] in comprehensive studies of the unsaponifiable fraction of wheat germ oil and of a very potent concentrate obtained from it. Only one of two oxygen atoms present could be acetylated, the other being inert, and the acetylated product was still active biologically. Reactions with iodine or hydrogen suggested the presence of three double bonds, but the concentrate resisted complete hydrogenation. Spectroscopic evidence, which is to be discussed, indicated that the hydroxyl group might be derived from a keto-enol structure but this view had to be abandoned later.[12] Data on surface pressures and surface potentials secured by Askew[13] suggested a reduced polycyclic structure of the sterol type.

A study of the curves or bands in the absorption spectrum of the ultraviolet region is an increasingly useful tool for elucidating organic structure. This method was first applied to crude concentrates of vitamin E by Morton and Bowden and their co-workers.[14] In the biologically active portion of much purer concentrates prepared from cottonseed as well as from wheat germ and palm oils Olcott[15] found a band with a maximum at 2,940 angstrom units; this was also confirmed.[16] His opinion that this band was not the property of the vitamin molecule but of some closely related impurity removable with difficulty, perhaps antioxidant,[9] was based on the lack of parallelism between the intensity of this band and the vitamin E content as determined biologically. Some effective concentrates showed no band; others, notably from palm oil, produced an intense band but had low biologic activity. Furthermore, various reagents caused the band to migrate without seeming to affect the vitamin. Despite further careful work on this question, Drummond and his co-workers[17] were compelled to leave it open.

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[11] Drummond, J. C.; Singer, Eleanor, and MacWalter, R. J.: A Study of the Unsaponifiable Fraction of Wheat Germ Oil with Special Reference to Vitamin E, Biochem. J. **29**: 456, 1935.

[12] Drummond, J. C.; Singer, Eleanor, and MacWalter, R. J.: Further Observations on the Constituents of the Unsaponifiable Fraction of Wheat Germ Oil with Particular Reference to Vitamin E, Biochem. J. **29**: 2510, 1935.

[13] Askew, Frederic: Surface Films of Vitamin E Concentrates, Biochem. J. **29**: 472, 1935.

[14] Bowden, Philip, and Moore, Thomas: Absorption Spectrum of the Vitamin E Fraction of Wheat-Germ Oil, Nature **131**: 512 (April 8) 1933. Morton, R. A., and Edisbury, J. R.: Absorption Spectrum of Unsaponifiable Matter from Wheat-Germ Oil, ibid. **131**: 618 (April 29) 1933. Bowden and Moore.[7]

[15] Olcott, footnotes 8 and 10.

[16] Martin, A. J. P.; Moore, Thomas; Schmidt, Marion, and Bowden, F. P.: Absorption Spectrum of Vitamin E, Nature **134**: 214 (Aug. 11) 1934.

Crystalline structure is usually considered a criterion of purity. All investigations thus far mentioned suffered from lack of purity of the starting material. This was a mixture; all the commonly used methods for isolating a substance in pure form had been unavailing. These included the use of varied solvents, high vacuum distillation which was often destructive, chromatographic absorption which Drummond found somewhat useful, and the preparation of crystallizable derivatives. Successful application of the last method on a microchemical scale with cyanic acid to combine with the hydroxyl group finally enabled Evans and the Emersons[18] to isolate three different crystalline allophanates from a vitamin E concentrate of wheat germ oil.[19] When the alcohols were regenerated from them, one proved to be inactive biologically, another, later called β-tocopherol, had some activity, and the third, which they called α-tocopherol, was effective in doses of from 1 to 3 mg. It was a light yellow viscous oil which resisted all attempts at crystallization, but its conversion to another crystalline derivative (with ρ-nitrophenyl isocyanate), followed by reconversion to the allophanate and regeneration of the alcohol, left its biologic activity unimpaired. The peak of its absorption band was at 2,980 angstrom units, and micro-analysis indicated a provisional formula, $C_{29}H_{50}O_2$. Soon thereafter they isolated the same α-tocopherol from cottonseed oil[20] and later[21] still another active substance, γ-tocopherol, from the same source. Further intensive investigations revealed that only α-tocopherol is present in lettuce and that palm oil is qualitatively similar to cottonseed oil and like it contains no β-tocopherol. These substances have unequal biologic activity, the α being more effective than β- or γ-components. They also proved to be antioxygenic[22] and hence possibly phenolic in nature, but the antioxygenic action did not parallel biologic activity, the γ being the most effective inhibitor. The confusion that has existed between the observations of different investigators is thus largely resolved, and the ultimate consequences of this resolution will be important. One may now speak of the multiple nature of vitamin E in much the same sense as we accept the fact that there are several substances which have vitamin D activity.

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[17] Drummond, Singer and MacWalter, footnotes 11 and 12.

[18] Evans, H. M.; Emerson, O. H., and Emerson, Gladys A.: The Isolation from Wheat Germ Oil of an Alcohol, α-Tocopherol, Having the Properties of Vitamin E, J. Biol. Chem. **113**: 319 (Feb.) 1936.

[19] This is the first record of the successful production of crystalline derivatives except for the preliminary note of Kimm (Kimm, Riang-Ha: Crystalline Derivatives of Vitamin E: Preliminary Report, Sc. Papers Inst. Phys. Chem. Research [Tokyo] **28**: 74, 1935), who from a concentrate of rice embryo oil obtained crystals of several β-naphthoic esters of vitamin E which gave modified sterol color reactions; the regenerated vitamin E had the empirical formula $C_{29}H_{48}O$ and was surprisingly potent biologically. Further important details are lacking and attempts to confirm his results have been unsuccessful (Todd, A. R.; Bergel, F.; Waldman, H., and Work, T. S.: Chemical Studies on Vitamin E: I. The Isolation of Some Crystalline Alcohols from the Unsaponifiable Matter of Rice and Wheat Germ Oils, Biochem. J. **31**: 2247 [Dec.] 1937).

[20] Emerson, O. H.; Emerson, Gladys A., and Evans, H. M.: Isolation from Cottonseed Oil of Alcohol Resembling Alpha Tocopherol from Wheat Germ Oil, Science **83**: 421 (May 1) 1936.

[21] Emerson, O. H.; Emerson, Gladys A.; Mohammad, A., and Evans, H. M.: The Chemistry of Vitamin E, J. Biol. Chem. **122**: 99 (Dec.) 1937.

In several other laboratories[22a] tocopherols were isolated from wheat germ oil notably by Drummond, Todd, (London), Karrer, (Zurich), and their coworkers and also by John (Göttingen). The last mentioned called his substance cumo-tocopherol, Karrer called his neo-tocopherol, and they are probably all identical with the β-tocopherol of Emerson. The preponderance of the alpha in the American wheat germ oils and of the beta in European oils is not explained.

The chemical identification of vitamin E began with the thermal decomposition product, duroquinone (tetramethyl hydroquinone), obtained from alpha tocopherol by Fernholz[23] (Merck's laboratory). By selenium dehydrogenation McArthur and Watson[24] also obtained it. Bergel, Todd and Work[24a] made these same observations on concentrates as well as on pure preparations, finding that beta tocopherol yields pseudocumoquinone

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[22] Olcott, H. S., and Emerson, O. H.: Antioxidants and the Autoxidation of Fats: IX. The Antioxidant Properties of the Tocopherols, J. Am. Chem. Soc. **59**: 1008 (June) 1937.

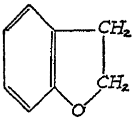
[22a] Drummond, J. C., and Hoover, A. A.: Studies on Vitamin E (Tocopherol), Biochem. J. **31**: 1852 (Oct.) 1937. Todd, A. R.; Bergel, F., and Work, T. S.: Studies on Vitamin E, II: The Isolation of β-Tocopherol from Wheat Germ Oil, Biochem. J. **31**: 2257 (Dec.) 1937. Karrer, P.; Salomon, H., and Fritsche, H.: Constituents of Plant Embryos, II: Neotocopherol, Helv. Chim. Acta **20**: 1422 (6) 1937. John, W.: Cumotokopherol, Ztschr. f. Physiol. Chem. **250**: 11 (No. 1) 1937. Karrer, P., and Salomon, H.: The Isolation of Tocopherols from Wheat Germ Oil, Helv. Chim. Acta **21**: 514 (3) 1938.

[23] Fernholz, E.: The Thermal Decomposition of α-Tocopherol, J. Am. Chem. Soc. **59**: 1154 (June) 1937.

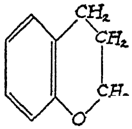
[24] McArthur, C. S., and Watson, E. M.: The Selenium Dehydrogenation of α-Tocopherol, Science **86**: 35 (July 9) 1937.

(trimethyl hydroquinone). John[22a] had also gotten pseudocumoquinone; hence the name he gave to his tocopherol.

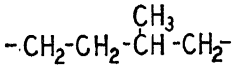
The suggestion that tocopherol might be a mono ether of duroquinone was shown to be untenable by John,[24b] Todd[24a] and Karrer[24c] and their co-workers, all of whom proposed a coumarane (a) or chromane (b) nucleus with a side chain (Karrer) of isoprene residues (c) as in phytol (the alcoholic portion of chlorophyll). Fernholz[24d] had also concluded from the dissimilarities of the absorption spectra of ethers of duroquinone and of tocopherol that this did not have a simple ether structure; from an examination of the chromic acid oxidation products of tocopherol and after some close and skillful organic chemical reasoning he proposed the following (chromane) formula for alpha tocopherol:



(a). Coumarane

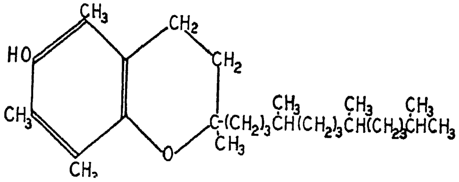


(b). Chromane



$$-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-$$

(c). Isoprene residue in side chain



Alpha tocopherol ("chromane" formula)

24a. Bergel, F.; Todd, A. R., and Work, T. S.: Vitamin E. III: Observations on the Structure of Alpha and Beta Tocopherol, J. Chem. Soc., 253 (Feb.) 1938.

24b. John, W.; Dietzel, E., and Günther, Ph.: Hydriodic Acid Cleavage of Tocopherols, Z. Physiol. Chem. **252**: 208 (5/6) 1938. John, W.: Constitution of Tocopherol, ibid. **252**: 222 (5/6) 1938.

24c. Karrer, P.; Salomon, H., and Fritsche, H.: Vitamin E, Helv. Chim. Acta. **21**: 309 (2) 1938.

24d. Fernholz, E.: Constitution of Alpha Tocopherol, J. Am. Chem. Soc. **60**: 700 (March) 1938.

Meantime, without yet having decided the question of chromane vs. coumarane, Karrer and his coworkers[24e] accomplished an almost quantitative condensation of trimethyl hydroquinone with phytyl bromide, zinc chloride being the catalyst. Except for optical activity the product had physical and chemical properties identical with those of naturally occurring vitamin E. Later[24f] this synthetic product was shown also to have the physiological properties of vitamin E and thus a unique event happened in organic-biochemical history—the synthesis of a complex substance before its actual constitution was known!

Intensive organic chemical work in two other laboratories had meanwhile confirmed the presence of a chromane nucleus. Excellent degradative studies by John and his coworkers[24g] demonstrated this nucleus with two substituents on carbon (optical activity). Smith and his coworkers[24h] at Minnesota, by still different organic chemical reasoning came to the same conclusion and succeeded in synthesizing alpha tocopherol by Karrer's method without a catalyst and by still another method, the products being thick viscous nearly colorless oils which had the physical, chemical and physiological[24i] properties of vitamin E.[24j] By permanganate oxidation Emerson[24k] also adduced proof for a chromane nucleus and more recently[24l] he proved that beta and gamma tocopherol differ from alpha only in the absence of one of the three methyl groups attached to the benzene ring, thus confirming earlier conclusions

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24e. Karrer, P.; Fritsche, H.; Ringier, B. H., and Salomon, H.: Alpha Tocopherol, Helv. Chim. Acta **21**: 520 (3) 1938.

24f. Karrer, P., and Demole, V.: Synthesis and Biological Assay of Vitamin E, Schweiz. med. Wchnschr. **68**: 954 (33) 1938. Karrer, P.; Fritsche, H.; Ringier, B. H., and Salomon, H.: Synthesis of Alpha Tocopherol, Helv. Chim. Acta **21**: 820 (4) 1938.

24g. John, W.; Dietzel, E.; Günther, Ph., and Emte, W.: The Chromane Structure of Alpha-Tocopherol Naturwissenschaften **26**: 366 (May 27) 1938.

24h. Smith, L. I.; Ungnade, H. E., and Prichard, W. W.: The Chemistry of Vitamin E. I. The Structure and Synthesis of a Tocopherol, Science **88**: 37 (July 8) 1938.

24i. Evans, H. M.; Emerson, Gladys, A., and Emerson, O. H.: The Chemistry of Vitamin E. II. Biological Assays of Various Synthetic Compounds, Science **88**: 38 (July 8) 1938.

24j. Some ethers of durohydroquinone (Fernholz, E., and Finkelstein, J., J. Am. Chem. Soc. **60**: 2402 [Oct.] 1938) were also shown to be biologically active[24i] if given in sufficiently large doses, 50-250 mg.

24k. Emerson, O. H.: The Chemistry of Vitamin E. III. Permanganate Oxidation of Alpha Tocopherol, Science **88**: 40 (July 8) 1938.

24l. Emerson, O. H.: The Structure of Beta and Gamma Tocopherols, J. Am. Chem. Soc. **60**: 1741 (Aug.) 1938.

(John[24m] and Karrer et al[24c]). Still more recently Karrer and his coworkers[24n] have submitted additional methods of synthesis and proofs of structure, and have developed a new method for quantitative assay, based on potentiometric measurements; this marks a great advance over the long and tedious biological method.

Thus another of the vitamins has been synthesized. A little more than 15 years have passed since the functional importance of vitamin E was first recognized; knowledge of its chemical constitution and its synthesis were achieved within the past twelve-month. In this consummation we have witnessed a more rapid succession of significant events than has ever been seen before in so short a time in any field of biochemical endeavor; this early success was due largely to the collaboration of organic chemists with their improved microchemical technique.

Now that synthetic tocopherols are available, their function and manner of action can henceforth be studied with greater accuracy and confidence.

PHYSIOLOGY AND PATHOLOGY

The progress of the chemical studies which have been outlined required continuous animal assays of the biologic activity of the products obtained. The most suitable method of assay uses adult female rats, properly prepared by subsistence on a diet deficient in vitamin E. Ability to bear living young following the administration by mouth of varying amounts of concentrates is a measure of vitamin E potency; when the dose is inadequate the embryos die and are resorbed.[25] There is considerable and thus far uncontrollable variability in the response of individual animals to the same dosage, and few statistical studies have been reported.[26] The reason for this is obvious: until recently the concentrates were all mixtures, and investigators were primarily interested in knowing the minimum effective dose of their preparations.

[24m] John, W.: The Identity of Cumo-Tocopherol and β-Tocopherol, Z. Physiol. Chem. **252**: 201 (5/6) 1938.

[24n] Karrer, P.; Escher, R.; Fritsche, H.; Keller, H.; Ringier, B. H., and Salomon, H.: Constitution and Assay of Alpha-Tocopherol and Some Similar Compounds, Helv. Chim. Acta **21**: 939 (5) 1938. Karrer, P., and Keller, H.: Quantitative Determination of Tocopherols in Various Natural Sources, ibid., p. 1161.

[25] A more detailed exposition of the method of assay is given in the previous review[1] and elsewhere.[6, 24f]

[26] Palmer, L. S.: Biological Assay of Vitamin E; Application to Wheat Germ and Wheat Germ Oil, Proceedings of the American Society of Biological Chemists **119**: lxxv (June) 1937; Indust. & Engin. Chem., Anal. Ed. **9**: 427 (Sept.) 1937.

The necessity of vitamin E for normal embryonic growth in animals other than the rat and the mouse has not been established, but several suggestive observations have been made in the field of animal production. The use of wheat germ oil or of natural foodstuffs has lessened the significance of the experiments as regards vitamin E itself. In the case of herbivorous animals, the very real difficulty of supplying a ration which is adequate in all respects save vitamin E has sometimes been met by treating the food with an ethereal solution of ferric chloride, thereby introducing still further complications.[5] Such a ration has been found to support normal growth and reproduction in goats and rabbits for several generations.[27] Thus seven original goats increased to forty-eight in fifty-four months with no reproductive disorders attributable to avitaminosis E. Twelve original rabbits littered 137 young in two years; there were infrequent reproductive disorders the cause of which is not yet ascertained.

The carefully controlled veterinary studies on cattle have been continued by Vogt-Møller.[28] Intramuscular or preferably subcutaneous injection of 20 cc. of "Fertilan",[29] a sterilized wheat germ oil, into otherwise normal cows that have repeatedly failed to become pregnant was followed by pregnancy in thirty-three out of fifty instances. Similar results were reported by Tutt.[30] Large doses of wheat germ oil increased the size of rabbit litters,[31] wheat germ meal in the diet of sows reduced the mortality of suckling pigs,[32] but this improvement may have been due to other constituents.

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27. Thomas, B. H., and Cannon, C. Y.: Reproduction on Rations Free from Vitamin E, Proc. Am. Soc. Animal Prod. **30**: 59 (Nov.) 1937, also personal communication from Dr. B. H. Thomas, Ames, Iowa.

28. Bay, F., and Vogt-Møller, P.: Continued Studies on Treatment of Sterility in Cows and Breeding Sows with Wheat Germ Oil (Vitamin E), Vet. J. **90**: 288, 1934.

29. Vogt-Møller, P.: On Estimation of the Vitamin E Content of the Wheat-Germ Oil Preparation "Fertilan" Mco., Acta path. et microbiol. Scandinav. **12**: 115, 1935.

30. Tutt, J. F., quoted by Bay and Vogt-Møller.[28]

31. Schioppa, Luigi: Experimental Researches on Vitamin E: IV. Researches on the Influence of Vitamin E on Fertility and the Bodily Condition of the Offspring, Ztschr. f. Vitaminforsch. **5**: 22 (Jan.) 1936.

32. Auble, C. E.; Hughes, J. S., and Lienhardt, H. F.: The Influence of Vitamins B and E on Reproduction in Swine, Proc. Am. Soc. Animal Production, 1929, p. 133.

The reported occurrence of vitamin E in the queen bee's royal jelly[33] could not be confirmed.[34]

The results obtained with chickens are more conclusive and trustworthy because the vitamin E content of the food of the hens and of their eggs as determined by rat assay can be correlated with the hatchability of the eggs and the viability of the young chicks.[35] From such experiments it appears that vitamin E may be a limiting factor in the hatchability of eggs and that one manifestation of its deficiency is a first-week embryonic mortality. This is greatly reduced by supplying vitamin E in the poultry ration. Agricultural journals are beginning to carry advertisements recommending the use of feeds enriched with biologically tested wheat germ oil. If there is any justification for this, it arises in part in the increasing tendency to introduce concentrates and already compounded rations into animal feeding practice. A shortage of vitamin E might result from the consumption of a more or less limited diet, depending on the nature of the constituents. It is conceivable that on more natural forage, which would ordinarily supply enough E, an occasional animal might exhibit symptoms of a shortage through faulty absorption[35a] or metabolism.

The embryonic development of chicks in eggs from hens on a vitamin E deficient ration has shed some light on the abnormalities causing death in the embryo. According to Adamstone,[36] cell proliferation in the mesoderm produces a lethal ring in the blastoderm, whose blood vessels are choked off and disintegrate;

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[33] Hill, Leonard, and Burdett, E. F.: Fertility of Bees and Vitamin E, Nature **130**: 540 (Oct. 8) 1932. The proper conduct of a biologic assay requires some experience; wheat germ oils are not alike in their vitamin E content and due care must be exercised in the preparation of concentrates (Evans, H. M.; Murphy, E. A.; Archibald, R. C., and Cornish, R. E.: Preparation and Properties of Vitamin E Concentrates, J. Biol. Chem. **108**: 515 [Feb.] 1935).

[34] Schoorl, P.: Vitamin E Research, Ztschr. f. Vitaminforsch. **5**: 246 (Oct.) 1936. Mason, K. E., and Melampy, R. M.: Absence of Vitamin E in the Royal Jelly of Bees, Proc. Soc. Exper. Biol. & Med. **35**: 459 (Dec.) 1936. Evans, H. M.; Emerson, Gladys A., and Ackert, J. E.: Alleged Vitamin E Content in Royal Jelly, J. Econ. Entom. **30**: 642, 1937.

[35] Barnum, G. L.: The Vitamin E Content of Eggs as Related to the Diet of the Hen and to Hatchability, J. Nutrition **9**: 621 (May) 1935. Ender, Fredrik: Significance of Vitamin E in Poultry Hus-

(May 20) 1937.

[35a] Greaves, J. D., and Schmidt, C. L. A.: Relation of Bile to Absorption of Vitamin E in the Rat, Proc. Soc. Exper. Biol. & Med. **37**: 40 (Oct.) 1937.

[36] Adamstone, F. B.: The Effects of Vitamin E Deficiency on the Development of the Chick, J. Morphol. & Physiol. **52**: 47 (Sept.) 1931.

the embryo dies from loss of blood and starvation. Observations on the degeneration in the testis of the male fowl[37] led him to conclude that vitamin E is intimately associated with the nucleus during cell division and probably exerts an indirect controlling influence.

Since the comprehensive morphologic studies made by Evans and Burr on the rat and described in their memoir, several workers have confirmed their observations. Thus Urner[38] found the embryo apparently normal until the tenth day, after which there was rarefaction of the mesenchyme and failure of the blood forming tissues. In the ectoplacenta also there was failure of the mesodermal elements. According to others,[39] the malnutrition of the embryos is due at first to the imperfect phagocytic action of the trophoblast and later to imperfect development of allantoic blood vessels.

The striking difference in response to vitamin E deprivation by male and female animals can be explained, superficially, by the fact that in the male the damage is done to a part of the animal's own tissue and may for this reason be irreversible in contrast to the reparable damage in the female, which is wrought not on her own tissues but on those of the fetus. Among the nutritional factors concerned in testicular degeneration, vitamins A and E are probably the most important. On diets low in A and containing abundant E, degeneration appears earlier than under reversed conditions.[40] At first believed to be very similar,[41] the histologic pictures presented by the two conditions are different, as demonstrated in a comprehensive study by Mason.[42] Deficiency of vitamin E produces excessive liquefaction of the chromatin material, first in the spermatozoa and spermatids and later in the less mature cells; spermatogenic activity ceases soon after the first appearance of injury, whereas in vitamin A deficiency it continues in spite of considerable depletion of the germinal epithelium.[43] A careful survey of all the facts available leads Mason to the conclusion that vitamin E plays some very essential rôle in nuclear activities involving chromatin and is indispensable, especially in those tissues in which cellular proliferation and differentiation are unusually rapid. Such are the testis in the male and the developing embryo in the female.

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[37] Adamstone, F. B., and Card, L. E.: Effects of Vitamin E Deficiency on the Testis of the Male Fowl (Gallus Domesticus), J. Morphol. **56:** 339 (Sept.) 1934. Adamstone, F. B.: Possible Relation of Vitamin E to Unrestricted Cell Division, Science **80:** 450 (Nov. 16) 1934.

[38] Urner, J. A.: The Intra-Uterine Changes in the Pregnant Albino Rat (Mus Norvegicus) Deprived of Vitamin E, Anat. Rec. **50:** 175 (Aug.) 1931.

[39] Zagami, V., and Sindoni, M.: Vitamin E Deficiency and Pregnancy in Rats, Riv. di pat. sper. **12:** 1 (March) 1934; through Physiol. Abstr. **20:** 756 (Jan.) 1936.

[40] Evans, H. M.: Testicular Degeneration Due to Inadequate Vitamin A in Cases Where E Is Adequate, Am. J. Physiol. **99:** 477 (Jan.) 1932.

[41] Korenchevsky, V.: Sterility in Males on Diets Deficient in Vitamin A and E, Proc. Roy. Soc. Med. **26:** 1187 (July) 1933.

[42] Mason, K. E.: Differences in Testis Injury and Repair After Vitamin A Deficiency, Vitamin E Deficiency and Inanition, Am. J. Anat. **52:** 153 (March) 1933.

A possible quantitative difference in the requirements of the two sexes [44] may emerge from this conception, the male requirement being greater than the female. This cannot be determined until larger amounts of the various tocopherols become available for study. Awaiting similar proof is the assumption of sex specific forms of vitamin E.[45]

Does vitamin E have any other functions in the animal economy beyond those concerned with reproduction? Its effect in increasing the number and viability of the young [46] is a common observation; vitamin E is probably not concerned with lactation. Its favorable influence on extra-uterine growth, first observed by Evans and by others,[42] was overlooked by some,[47] perhaps because their experiments were of too short duration. Interest was renewed in the subject by Blumberg[48] and by Ringsted.[49] The former demonstrated a retardation of growth in young rats at the twelfth to the fourteenth week (in males from the very beginning), complete cessation of growth at from eighteen to twenty-two weeks, and serious malnutrition and some muscular disturbance at from thirty to forty weeks when the animals were maintained on a highly purified ration deficient in vitamin E. Addition to the diet of wheat germ, the unsaponifiable fraction of its oil, or of natural foods known to contain E, produced a resumption of growth whereas allied substances known not to contain E had no effect. Ringsted's animals (females) reached a plateau of growth and first showed dragging of the hind limbs at 160 Gm. and at from 5 to 6 months of age; the paresis and malnutrition gradually increased on his synthetic diet, which contained lard previously aerated for ten hours at 110 C. and which was thus very rancid. Unfortunately he was unable to try curative procedures, but animals on the same diet that had received test doses of wheat germ oil adequate for successful reproduction remained free of paralysis.

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[43] Acccording to C. A. Pfeiffer (Some Factors Influencing the Vitalization of the Ovarian Graft and the Production of Sex Hormones in the Male Rat, Endocrinology **21**: 260 [March] 1937) diphtheria toxin produces a rapid degeneration of the germinal epithelium similar to that of vitamin E deprivation, except that it is reversible.

[44] Waddell, James; Steenbock, Harry, and Hart, E. B.: Growth and Reproduction on Milk Diets, J. Nutrition **4**: 53 (May) 1931. Waddell, James: Male Sterility on Milk Diets, J. Nutrition **4**: 67 (May) 1931. Keil. H. L., and Nelson, V. E.: Preservation of Fertility in Male and Female Rats on a Supplemented Milk Diet, Proc. Soc. Exper. Biol. & Med. **33**: 490 (Jan.) 1936.

[45] Grijns, G., and Dingemanse, E.: Diet and Reproduction: IV. The Bipartite Nature of Vitamin E, Proc. Acad. Sc. Amsterdam **36**: 242, 1933; through Chem. Abstr. **27**: 3974 (Aug. 20) 1933. Martino, G.: The Dual Nature of Vitamin E, Quaderni nutrizione **1**: 180, 1934; through Chem. Abst. **30**: 4546 (July 10) 1936. Cunningham, I. J., and Hopkirk, C. S. M.: Dietary Protein in Relation to Sterility, New Zealand J. Sc. Tech. **17**: 420 (No. 1) 1935; through Chem. Abstr. **30**: 142 (Jan. 10) 1936. Martino, G., and Knallinsky, A.: The Vitamin E Content of the Manioc Root, Boll. soc. ital. biol. sper. **8**: 819, 1933; through Chem. Abstr. **29**: 3005 (May 10) 1935. Arthus, A.: Feeding Rats Exclusively with Milk Powders of the Same Origin but of Different Butterfat Contents, Bull. Soc. scient. d'hyg. aliment. **24**: 218, 1936; through Chem. Abstr. **30**: 7639 (Nov. 10) 1936.

[46] Schioppa, Luigi: Vitamin E: III. Influence of Vitamin E on Normal Animals, with Respect to Fertility, Size of Litter, and Somatic Characters, Ztschr. f. Vitaminforsch. **4**: 167 (July) 1935.

[47] Hogan, A. G.; Hunter, J. E., and Shrewsbury, C. L.: The Relation of Diet to Bodily Activity and to Capacity to Withstand Unfavorable Circumstances, Bull. 256, University of Missouri Experiment Station, 1927, p. 50. Marchesi, Franco: Vitamin E and Reproductive Function in Albino Rats, Sperimentale, Arch. di biol. **89**: 119, 1935; through Chem. Abstr. **29**: 5895 (Sept. 10) 1935.

Later work confirmed the growth promoting effect of vitamin E but without demonstrating any paralysis when this was withheld. Martin[50] exactly duplicated the basal diet and management that Blumberg used; the weights when growth ceased were slightly higher in both sexes, and the giving of a vitamin E concentrate restored growth. Attempts by various means to segregate a growth factor and a fertility factor in the concentrate led him to suggest that there were two agencies rather than a quantitative difference between the required amounts of a single substance. The total duration of his experiments is not indicated.

In a further study by Emerson and Evans,[51] female rats on an E-deficient diet were surpassed by controls only after 4 to 5 months of age and at 250 Gm. in weight, and massive doses of wheat germ oil restored growth. At 19 months of age untreated animals had an unsteady gait but no paralysis. Olcott and Mattill [52] also found no difference at 4 months between female animals on a synthetic E-deficient diet in which all the lipid constituents were of known composition, whether they were given a highly purified and tested concentrate of E or not. Male animals, on the other hand, were surpassed by controls from the age of 2 months; at 5 months the latter were 10 per cent heavier, but the former continued to grow and there was no paralysis. In both of these investigations an increased fat content (lard or ethyl esters of the fatty acids of edible fats) in the diet had a temporary stimulating effect on growth. Probably none of these studies was continued for a long enough time, for recently Burr and his co-workers [53] reported paralysis of the hind limbs in 22 months old female rats that had been on a highly purified E-deficient diet from weaning.

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[48] Blumberg, Harold: A Growth Deficiency Disease, Curable by Wheat Germ Oil, J. Biol. Chem. **108:** 227 (Jan.) 1935.

[49] Ringsted, Axel: A Preliminary Note on the Appearance of Paresis in Adult Rats Suffering from Chronic Avitaminosis E, Biochem. J. **29:** 788, 1935.

[50] Martin, G. J.: Vitamin E, J. Nutrition **13:** 679 (June) 1937.

[51] Emerson, Gladys A., and Evans, H. M.: The Effect of Vitamin E Deficiency upon Growth, J. Nutrition **14:** 169 (Aug.) 1937.

Equally mysterious is the paralysis in suckling rats from vitamin E-deficient mothers. First observed and studied by Evans, its incurable nature and its prevention by wheat germ oil and vitamin E concentrates have been confirmed repeatedly.[54] This condition was originally thought to be of nervous origin,[55] but it has now been definitely associated with skeletal muscles.[56] The muscles exhibit extensive degeneration and necrosis and their microscopic changes are not to be distinguished from those of muscular dystrophy in herbivora discussed later in this report; less extensive lesions may be present in the musculature of animals showing no external symptoms of paralysis or weakness.

The serious paralysis in first generation adult rats (Ringsted) was associated with a diet containing very rancid fat. The antioxygenic effect of the tocopherols and other inhibitors has up to now been considered as limited to the dietary mixture in which they are found and not as extending into the organism and its physiologic processes. Possibly the prolonged exposure of tissues to autoxidizing fatty acids and their products is as damaging to their chemically sensitive constituents as it is to the autoxidizable constituents of experimental diets. Support for this suggestion is found in the observations of Waddell and Steenbock[57] to the effect that on a ration treated with ferric chloride female rats were unable to use even large stores of vitamin E and did not acquire this ability until from four to six weeks after transfer to a nonoxidized basal ration. A further interesting item has recently come to notice;[58] subcutaneous injection (0.1 Gm.) of a concentrate prepared from rancid fat and containing aldehydes and ketones produced resorption of the embryos or failure of implantation[58a] and fertility rapidly returned on discontinuance of the injections. Confirmation and interpretation of these observations will be significant for a proper understanding of the functions of vitamin E and its allied substances.

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[52] Olcott, H. S., and Mattill, H. A.: Vitamin E and Growth, J. Nutrition **14:** 305 (Sept.) 1937.

[53] Burr, G. O.; Brown, W. R., and Moseley, R. L.: Paralysis in Old Age in Rats on a Diet Deficient in Vitamin E, Proc. Soc. Exper. Biol. & Med. **36:** 780 (June) 1937.

[54] Morelle, Jean: Influence of Deprivation of Vitamin E on Lactation in the Rat, Compt. rend. Soc. de biol. **108:** 804 (Nov. 27) 1931. Olcott and Mattill.[6] Mason.[42]

[55] Lipschutz, Daniel: Degeneration of Nerve Tracts in Young Rats Lacking Vitamin E, Rev. neurol. **65:** 221 (Feb.) 1936.

[56] Olcott, H. S.: Paralysis in the Young of Vitamin-E Deficient Female Rats, Proceedings of the American Society of Biological Chemists **119:** lxxiv (June) 1937. J. Nutrition **15:** 221 (March) 1938.

The relation of these various instances of paralysis to the muscular dystrophy first observed in herbivorous animals by Goettsch and Pappenheimer[59] is not clear. The latter type of dystrophy was first produced in guinea pigs and rabbits, even in utero,[60] on a diet deficient in vitamin E, but the addition of this to the diet did not prevent the development of the disease. On the same deficient diet, rats did not manifest the disease. According to Madsen, McCay and Maynard,[61] cottonseed oil (containing a concentrate of vitamins A and D) in place of cod liver oil afforded a high degree of protection, and one of the causative factors resided in the saponifiable fraction of cod liver oil. Recently

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57. Waddell, James, and Steenbock, Harry: Vitamin E in Iron Treated Dry Rations, J. Nutrition **4:** 79 (May) 1931.

58. Kudryashov, B. A., and Agatov, P. A.: Sterilization by Means of Decomposed Fat, Ginekologia i Akusherstvo **6:** 1, 1935; through Chem. Abstr. **31:** 1873 (March 20) 1937.

58a. In this connection see also Bacharach, A. L.; Allchorne, Edith, and Glynn, H. E.: The Influence of Vitamin E Deficiency on Implantation, Biochem. J. **31:** 2287 (Dec.) 1937; ibid. **32:** 1298 (Aug.) 1938.

59. Goettsch, Marianne, and Pappenheimer, A. M.: Nutritional Muscular Dystrophy in the Guinea Pig and Rabbit, J. Exper. Med. **54:** 145 (Aug.) 1931. Pappenheimer, A. M., and Goettsch, Marianne: Nutritional Myopathy in Ducklings, ibid. **59:** 35 (Jan.) 1934.

60. Pappenheimer, A. M., and Goettsch, Marianne: Transmission of Nutritional Muscular Dystrophy to Rabbits in Utero, Proc. Soc. Exper. Biol. & Med. **34:** 522 (May) 1936.

61. Madsen, L. L.; McCay, C. M., and Maynard, L. A.: Synthetic Diets for Herbivora with Special Reference to the Toxicity of Cod Liver Oil, Bull. 178, Cornell University Agricultural Experimental Station, 1935, p. 3; Proc. Soc. Exper. Biol. & Med. **30:** 1434 (June) 1933. Madsen, L. L.: The Comparative Effects of Cod Liver Oil, Cod Liver Oil Concentrate, Lard and Cottonseed Oil in a Synthetic Diet on the Development of Nutritional Muscular Dystrophy, J. Nutrition **11:** 471 (May) 1936.

McCay, Paul and Maynard[62] reported that the untoward effects produced by cod liver oil in the diet of herbivora were abolished when hydrogenated cod liver oil replaced the natural product. Muscle lesions in guinea pigs were entirely prevented by this substitution. Fats that easily become rancid are greatly stabilized by hydrogenation.[63]

The experiments of Morgulis and co-workers[64] indicate that at least two factors are involved in this muscular degeneration; one of them is closely associated or perhaps identical with vitamin E since it occurs in lettuce, alfalfa and the vegetable oils as well as in their nonsaponifiable portion; the other is water-soluble and belongs to the vitamin B complex (perhaps B_4) and is present in wheat germ, lettuce and yeast. The value of their metabolic and blood studies[65] is limited by the fact that inanition accompanies the dystrophy. The oxygen consumption of these degenerated muscles seems to be elevated,[66] but the metabolic rate of the afflicted animals is normal.[67]

Whether one or two factors may be concerned, it has recently been suggested[67a] that autoxidative rancidity of the animal fats included in dystrophy-producing diets may be the principal cause of the disorder; vitamin E is antioxygenic in these fats[22] and therefore it disappears in their presence[4] even before rancidity becomes evident. Herbivorous animals have a large cecum where the food remains long enough for autoxidative changes to progress farther and more rapidly than in omnivorous animals such as rats. From this point of view the long search for a toxic factor in cod liver oil, and for cures of the disorders produced thereby (cattle, rabbits, poultry) may have been following a wrong trail.

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[62] McCay, C. M.; Paul, Henry, and Maynard, L. A.: The Removal by Hydrogenation of the Properties of Cod Liver Oil Which Are Harmful to Herbivora, read before the meeting of the American Chemical Society in Rochester in September 1937.

[63] Nutritional encephalomalacia in chicks was completely prevented by including various vegetable oils in the diet (Pappenheimer, A. M., and Goettsch, Marianne: Protection Afforded by Certain Vegetable Oils Against Nutritional Encephalomalacia in Chicks, Proc. Soc. Exper. Biol. & Med. **31:** 777 [April] 1934). Vegetable oils contain inhibitols;[9] animal fats do not.

[64] Morgulis, Sergius, and Spencer, H. C.: A Study of the Dietary Factors Concerned in Nutritional Muscular Dystrophy, J. Nutrition **11:** 573 (June) 1936; Morgulis, S.; Wilder, Violet M., and Eppstein, S. H., ibid. **16**:219 (Sept.) 1938.

[65] Morgulis, Sergius, and Spencer, H. C.: Studies on the Blood and Tissues in Nutritional Muscular Dystrophy, J. Nutrition **12:** 173 (Aug.) 1936; Metabolism Studies in Nutritional Muscular Dystrophy, ibid. **12:** 191 (Aug.) 1936.

[66] Victor, Joseph: Metabolic and Irritabilty Changes in Nutritional Myopathy of Rabbits and Ducks, Am. J. Physiol. **108:** 229 (April) 1934. Madsen.[61]

[67] Wood, E. L., and Hines, H. M.: Effect of Vitamin E Deficient and Muscular Dystrophy-Producing Diet on Metabolism of Guinea Pigs, Proc. Soc. Exper. Biol. & Med. **36:** 746 (June) 1937.

[67a] Mattill, H. A.: Vitamin E and Nutritional Muscular Dystrophy in Rabbits, Proc. XVI Intern. Physiol. Cong. **2:** 112 (Aug.) 1938.

What value these observations may have for the understanding of human muscular dystrophy should be and is doubtless already a subject for careful inquiry.

A functional relation between vitamins and internal secretions has been an intriguing if not very fruitful idea. Naturally, a possible connection of vitamin E with the sex hormones came to mind. It was Verzár [68] who first stated that vitamin E acted like anterior hypophysial hormone in inducing precocious sexual maturity in young female rats but not in castrates and that E might be necessary for the formation of the hypophysial hormone. Szarka [69] even concluded that E might be a building stone or precursor of the ovarian hormone, but since estrus is usually normal in vitamin E deficiency so direct a relationship is physiologically inadmissible. These views have found experimental contradiction in the work of many investigators; [70] vitamin E does not exhibit gonadotropic or luteinizing effects, nor do extracts of anterior pituitary, or pregnancy urine or extracts of corpus luteum prevent reproductive failure in rats on vitamin E-deficient diets.

Although recently denied,[71] there seems no doubt that, as first noted by van Wagenen,[72] cellular changes take place in the anterior hypophysis of vitamin E-deficient male animals; [73] so-called castration cells

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68. Verzár, Friedrich: The Influence of Diet on Internal Secretion, Proc. Staff Meet., Mayo Clin. **4:** 351 (Dec. 4) 1929.

69. Szarka, A.: The Action of E on Ovarian Function: XI. Estrus and Avitaminosis, Arch. f. d. ges. Physiol. **223:** 657 (Dec. 10) 1929.

70. Csik, Lajos: Mechanism of the Action of Vitamin E: Action of Menformone, Magyar Biol. Kutato Intezet Munkai **5:** 179, 1932; through Chem. Abstr. **27:** 2183 (May 10) 1933. Kudryashov, B. A.: Vitamins and the Female Sex Hormone: Is Vitamin E Necessary for the Synthesis of the Female Sex Hormone in Females? Dynamics Development (U. S. S. R.) **10:** 37, 1935; through Chem. Abstr. **30:** 4204 (June 20) 1936. Diakov, F. A., and Krizenecky, J.: Vitamin E and Pituitary Hormone: I and II, Proc. Soc. Exper. Biol. & Med. **31:** 59 (Oct.) 1933. Saphir, William: Vitamin E and the Gonads, Endocrinology **20:** 107 (Jan.) 1936. Geller, F. C.: Vitamin E and Hormones, Arch. f. Gynäk. **156:** 345 (Dec.) 1933. Nelson, W. O.: Studies on the Anterior Hypophysis: III. The Anterior Hypophysis in Vitamin E-Deficient Rats, Anat. Rec. **56:** 241 (June) 1933. Olcott and Mattill.[6]

71. Müller, J. H., and Müller, C.: Morphological Changes in the Adenohypophysis of Rats on a Vitamin E-Free Diet, Endokrinologie **18:** 369 (May) 1937; through Chem. Abstr. **31:** 5025, 1937.

72. van Wagenen, Gertrude: Histological Changes in the Male Rat Hypophysis Following Degeneration of the Germinal Epithelium, Anat. Rec. **29:** 398 (March) 1925.

73. Stein, S. I.: Experimental Studies on the Hypophysis Cerebri: IV. The Effect of Vitamin E Deficiency in the Female Albino Rat. J. Nutrition **9:** 611 (May) 1935. Geller.[70] Nelson.[70]

appear, which are enlarged vacuolated basophile cells, but not of the signet ring type observed in surgical castrates. The condition is more marked in castrates than in vitamin E-deficient males; the glands of vitamin E-deficient female animals remain normal. E-deficient male pituitaries are more potent in the gonad-stimulating hormone than glands from normal animals and less potent than those of castrates, whereas E-deficient female glands do not differ from normal. This sex-different response in the pituitary would support the belief that the changes observed are directly the result of gonadal degeneration and are only indirectly concerned with vitamin E. The same significance probably attaches to the progressive hypertrophy of the mammary glands of vitamin E-deficient rats throughout a resorption gestation.[74]

More recently however, biologic assay of pituitaries of E-free female rabbits by Rowlands and Singer [75] has given contrary results; such pituitaries demonstrated a decreased content of the luteinizing or ovulation-producing substance that is active in the rabbit, but such deficiency could not be considered a primary cause of the fetal resorption. The finding of hypoplastic thyroids in female E-deficient rats,[76] with return to normal appearance on administering E, suggests that thyrotropic as well as gonadotropic activity of the pituitary may be altered in the absence of vitamin E. Cretinism in young E-deficient rats has also been reported.[77]

Interest in the possible relationship of vitamin E to malignancy was awakened by Davidson's observations on tar cancer in susceptible mice.[78] The incidence of such cancer was delayed and less frequent, and the animals remained in health longer, on an E-containing diet than on one deficient in it. Davidson acknowledged that the E-containing diet was better in other respects also, notably in vitamins B_1 and B_2. Earlier observations of Zagami[79] on the growth of grafts of Jensen sarcoma in rats demonstrated no significant differences as between animals with and without E in the diet. In the hands of Haddow and Russell,[80] liberal addition of wheat germ to the diet had no influence on the subsequent emergence of benzpyrene tumors, and unpublished work from this laboratory[81] on the incidence and metastasis of methyl cholanthrene cancers in both ordinary and susceptible mice is to the same effect. On the other hand, Adamstone[82] found that the feeding of a ferric chloride treated diet to young chicks caused the development of characteristic visceral lesions, lymphoblastomas, with destruction of normal tissue and invasion by new cell growths. The uncertainty of the character of ferric chloride damage forbids any conclusions at this stage of study.[83] In this connection two unconfirmed reports should be mentioned; the healing of skin wounds in rats is hastened by a diet rich in E[84] or by external application of a concentrate.[85]

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[74] Urner, J. A.: The Changes in the Mammary Glands of Pregnant Albino Rats Deprived of Vitamin E, Sect. Obst., Gynec. & Abd. Surg., A. M. A., 1930, p. 34.

[75] Rowlands, I. W., and Singer, Eleanor: Gonadotropic Activity of the Pituitaries of Vitamin E Deficient Rats, J. Physiol. **86:** 323 (March) 1936.

[76] Singer, Eleanor: Effects of Vitamin E Deficiency on the Thyroid Gland of the Rat, J. Physiol. **87:** 287 (Aug.) 1936.

[77] Barrie, M. M. O.: Effect of Vitamin E Deficiency on the Thyroid, Nature **139:** 286 (Feb. 13) 1937.

[78] Davidson, J. R.: An Attempt to Inhibit the Development of Tar Carcinoma in Mice, Canad. M. A. J. **31:** 486 (Nov.) 1934; **32:** 364 (April) 1935.

THERAPY

Definite proof for the value of vitamin E as a remedy for sterility in human beings is manifestly difficult to secure. Many causes and conditions have been shown to be associated with human sterility, and, so far as animal experiments indicate, vitamin E is directly concerned with only one phase of the female reproductive process, namely, the blood supply and nutrition of the embryo, and in the male with the maintenance of testicular function. By analogy, an inadequacy of vitamin E in the human male might therefore be revealed either by nonviability or by complete absence of sperm; in the female by abortion, the physiologic counterpart of resorption in rats. Such analogies are unsafe as between animals of different species, for they may imply too much or too little.

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[79] Zagami, V.: Effect of Vitamin E on Development of Rat Sarcoma, Riv. di pat. sper. **11:** 381 (Dec. 31) 1933; through Physiol. Abstr. **19:** 348 (Aug.) 1934.

[80] Haddow, Alexander, and Russell, Helen: The Influence of Wheat-Germ Oil in the Diet on the Induction of Tumors in Mice, Am. J. Cancer **29:** 363 (Feb.) 1937.

[81] Carruthers, Christopher: Unpublished data.

[82] Adamstone, F. B.: A Lymphoblastoma Occurring in Young Chicks Reared on a Diet Treated with Ferric Chloride to Destroy Vitamin E, Am. J. Cancer **28:** 540 (Nov.) 1936.

[83] Sufficient opportunity and time have not yet been afforded for verification of a striking announcement recently made by Rowntree and his co-workers (Rowntree, L. G.; Steinberg, A.; Dorrance, G. M., and Ciccone, E. F.: Sarcoma in Rats from the Ingestion of a Crude Wheat Germ Oil made by Ether Extraction, Am. J. of Cancer **31:** 359 [Nov.] 1937). When rats on an ordinary stock ration were fed a crude preparation of ether-extracted wheat germ oil, malignant tumors developed in the peritoneal cavity. They had the characteristics of spindle cell sarcoma and retained their malignancy through successive transplantations. Various preparations of refined wheat germ oil, extracted or expressed, and other vegetable and animal oils had no such effect. Whatever the agent may be, it is not vitamin E.

[84] Pegreffi, Enrico: Influence of Vitamin E on Healing of Skin Wounds, Pathologica **27:** 543 (Aug. 15) 1935.

[85] Léránth, Géza, and Frank, László: Significance of Vitamin E in Dermatology, Orvosi hetil. **80:** 778 (Aug. 15) 1936; through Chem. Abstr. **30:** 7634, 1936.

Early observations[1] on the value of vitamin E in habitual abortion have been continued and amplified. Vogt-Møller[86] has reported two series of twenty and fifty-two cases in which no anatomic or physiologic abnormalities could explain repeated abortion. Administration of 3 Gm. daily of a purified wheat germ oil preparation resulted in the birth of living children in seventeen and thirty-eight cases, respectively. Watson and Tew[87] reported success in thirty-four of forty-six cases of previous abortion; wheat germ oil may also be useful as an adjunct in threatened abortion but is of no avail in the treatment of nonfertility from other causes. The success of Currie[88] seems to have been still more pronounced. By administering daily from the time of first attendance to the onset of labor a 3 minim (0.2 cc.) capsule containing a concentrate (Glaxo) from 5 Gm. of wheat germ oil, he secured twenty-three normal births out of twenty-four cases in which the aggregate seventy-three previous pregnancies had resulted in only 14 per cent of living children. Cromer[88a] reports 7 cases in which recurrent or threatened abortion was prevented by administering large amounts of vitamin E.

Massive doses of wheat germ oil have also, according to Shute,[89] been useful in abruptio placentae and have prevented progress of these cases to a severe stage of placental detachment. This clinical experience fol-

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86. Vogt-Møller, P.: Treatment of Sterility and Habitual Abortion with Wheat Germ and Oil of Wheat Germ (Vitamin E), Hospitalstid. **76:** 621 (June 1) 1933; Acta obst. et gynec. Scandinav. **13:** 219, 1933; The Treatment of Patients Subject to Chronic Abortion with Wheat-Germ Oil (Vitamin E), Klin. Wchnschr. **15:** 1883 (Dec. 19) 1936.

87. Watson, E. M., and Tew, W. P.: Wheat Germ Oil (Vitamin E) Therapy in Obstetrics, Tr. Am. A. Obst., Gynec. & Abd. Surg. **48:** 189, 1935. Watson, E. M.: Clinical Experiences with Wheat Germ Oil (Vitamin E), Canad. M. A. J. **34:** 134 (Feb.) 1936.

88. Currie, D. W.: Vitamins for Habitual Abortion, Brit. M. J. **1:** 752 (April 11) 1936.

88a. Cromer, J. K.: Wheat Germ Oil (Vitamin E) in the Treatment of Repeated Spontaneous Threatened Abortion, Med. Ann. Dist. Columbia **7:** 145 (May) 1938.

89. Shute, Evan: Early Diagnosis of Abruptio Placentae and Its Treatment with Wheat Germ Oil, Am. J. Obst. & Gynec. **33:** 429 (March) 1937.

lowed the observation [90] that the blood serum of aborting women had an increased resistance to tryptic proteolysis and that the serum of rats acquired the same property after about four months on a vitamin E-deficient diet; normal digestibility was restored in both cases by administering vitamin E. There is as yet no physiologic explanation for this.

More clinical evidence, obtained under carefully controlled conditions, is greatly needed to establish the usefulness of vitamin E therapy in abnormal human reproduction. Until this is at hand, attempts to produce a market for wheat germ oil among prospective parents generally are to be deprecated; so also is the suggested threat of national dietary sterility, in view of the widespread distribution of vitamin E in the foods belonging in a well balanced diet. Individual cases of inadequacy, due perhaps to faulty absorption or metabolism will not be understood until more is known about the physiology of the "tocopherols."

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[90] Shute, Evan: Resistance to Proteolysis Found in the Blood Serum of Aborting Women, J. Obst. Gynaec. Brit. Emp. **42**: 1071, 1085 (Dec.) 1935; Relation of Deficiency of Vitamin E to Antiproteolytic Factor Found in Serum of Aborting Women, ibid. **43**: 74 (Feb.) 1936.

CHAPTER XXXI

OTHER FACTORS

LESS WELL KNOWN VITAMINS

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ITHACA, N. Y.

Under the title of "Other Factors" one can include only a few of the many essential organic substances that various animal species seem unable to synthesize. The description of these substances is scattered throughout the vast literature of biology. Many more have probably been missed than have been found. Such substances may seem of little importance at this time, but who can say when the needs of some insect with specific requirements may provide the key for studying the vitamin physiology of higher species? These ramifications of nutrition are well illustrated by the discovery of Trager and co-workers that a factor essential for growth of mosquito larvae is present in normal human urine but deficient in that of pernicious anemia patients.

To appreciate the expanse of unexplored fields in the domain of nutrition, one need merely read some modern work such as "Culture Methods for Invertebrate Animals" by Needham.[1] All recent tests with insects indicate that they require special factors that have not been described thus far as essential for higher forms. Today one can only postulate that, as higher forms such as man lost their fins and acquired hands in the course of evolution, they also abandoned some of their primitive vitamin needs and acquired still others.

A detailed discussion of insect nutrition is not appropriate in this paper, but passing consideration is worth while. Some of the most primitive of insects, such as the common cockroach, require constituents of yeast such as the vitamin B fractions as well as other factors which still remain undescribed.[2] Crowell and McCay[3] found that even clothes moths require some of the fractions of yeast. No clear-cut evidence has ever appeared, on the other hand, which indicates that any insect species requires either vitamin A or D, although certain lipid materials, possibly sterols, seem essential to such forms as the cockroach. As the insect field is explored further it is likely that needs for the fat soluble vitamins may appear.

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[1] Needham, J. G.: Culture Methods for Invertebrate Animals, Ithaca, N. Y., Comstock Publishing Company, 1937.

[2] McCay, C. M.: An Insect Test for Vitamin B Fractions, Proc. Am. Soc. Biol. Chemists **100:** lxvii, 1933; The Nutritional Requirements of Blatella Germanica, Physiol. Zool. **9:** 89-103 (Jan.) 1938.

[3] Crowell, Mary F., and McCay, C. M.: Nutritional Studies of the Webbing Clothes Moth, Tineola bisselliella Hum., Physiol. Zool. **10:** 368, 1937.

Trager,[4] in discussing the nutrition of mosquito larvae, concluded that there must be certain compounds other than the recognized vitamins that are essential. Frost and others found that mosquito larvae need certain essentials in yeast but not vitamin A, B, C, D or E. Fortunately, students of insect nutrition have recognized the limitations in the alphabet and have not attempted to label their discoveries with letters and subscripts. Such consideration for the future of the science of nutrition, however, must not be rewarded by neglect of their discoveries.

If one considers still lower forms than insects, one must enter the field of bacterial nutrition, where other parts of the alphabet, such as V and X, have been used to describe specific factors. Knight [5] has provided an excellent summary of the nutrition of bacteria with considerable space devoted to vitamin factors. The possible usefulness of even the molds in assaying the vitamins is forecast in the recent study of Meiklejohn.[6]

The minute amount of growth stimulants with which the nutrition student of the future will have to be concerned is suggested in the recent studies of Kögl,[7] who has isolated a factor for yeast which acts in a concentration of one part in four hundred billion. The comparative activity of some new and old compounds are shown in the accompanying table, from Kögl.

Little attention has been devoted to invertebrates other than the insects. However, Wulzen [8] and her associates have devoted many years to the study of planaria. In her earliest studies Wulzen recognized that she was dealing with specific thermolabile substances that differ from the vitamins previously described. In recent attempts to isolate these factors, Greenberg and Schmidt[9] have concluded that they can be extracted from liver by ether but that they are not identical with either the phosphatids or the nonsaponifiable lipids. Bahrs[10] has found marked differences in the growth promoting power of the rabbit's intestine when fed to planaria, depending on such factors as age and diet of the rabbit. This opens a fertile field of investigation, planaria being used to assay tissues of higher forms.

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[4] Trager, William: On the Nutritional Requirements of Mosquito Larvae Aedes Aegypti, Am. J. Hyg. **22**: 475-493 (Sept.) 1935; A Growth Factor Required by Mosquito Larvae, J. Exper. Biol. **14**: 240-251 (April) 1937; Trager, W.; Miller, D. K., and Rhoads, C. P.: The Absence from the Urine of Pernicious Anemia Patients of a Mosquito Growth Factor Present in Normal Urine, J. Exper. Med. **67**: 469-480 (March) 1938.

[5] Knight, C. J.: Bacterial Nutrition, Report 210, Medical Research Council, London, 1936.

[6] Meiklejohn, A. P.: The Estimation of Vitamin B in Blood by a Modification of Schopfer's Test, Biochem. J. **31**: 1441 (Sept.) 1937.

[7] Kögl, F.: Active Principles and Plant Growth, Naturwissensch. **29**: 465, 1937.

[8] Wulzen, Rosalind: The Effect of Heating on Certain Foods, Univ. California Publ., Physiol. **7**: 1, 1926.

Comparative Activity of Several Compounds

Material	Organism	Dilution	Effect
Biotin.................	Yeast	1:400,000,000,000	Growth
Biotin.................	Yeast	1: 40,000,000,000	Growth
Auxin A...............	Carrot	1: 10,000,000,000	Retardation
Auxin A...............	Oats	1: 1,000,000,000	Curvature
Thyroxine............	Tadpole	1: 5,000,000,000	Metamorphosis
Epinephrine..........	Frog's eye	1: 20,000,000	Expansion

FACTOR H

The lower vertebrates, with the exception of fish, have been given little attention by students of nutrition. For the past period of nearly a century during which trout have been domesticated, it has been known that the various species require some factor present in fresh meat. After a series of studies in which trout were fed synthetic diets of purified constituents supplemented with yeast, tomato juice and cod liver oil, McCay, Bing and Dilley[11] found that trout could not live unless fed a small amount of fresh meat. This component was termed "factor H." In a series of later studies it was found to be thermolabile and readily destroyed in the course of ordinary drying. However, part of it was preserved when such sources as liver were dried in vacuo in inert gas. This substance is present in all fresh body tissues that have been studied. It seems to be the dominant factor in the nutrition of trout. The literature concerning this factor has been reviewed recently.[12] This factor seems to be essential for carnivorous species such as the fox and the mink. In many respects factor H resembles the thermolabile substance that Wulzen has found essential for planaria.

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[9] Greenberg, L. D., and Schmidt, C. L. A.: Studies on the Properties of a Growth Promoting Factor for Planaria Maculata, J. Exper. Zool. **73**: 375, 1936.

[10] Bahrs, Alice M.: The Modification of the Normal Growth-Promoting Power, for Planarian Worms, of the Digestive Mucosa of the Rabbit Under Variations in Diet, Fasting and Age, Physiol. Zool. **4**: 189-203, 1931.

[11] McCay, C. M.; Bing, F. C., and Dilley, W. E.: Factor H in the Nutrition of Trout, Science **67**: 249 (March 2) 1928.

The letter H has also been applied to a totally different substance, which Booher [13] finds essential for rats. This factor is said to be heat stable but destroyed by alkali. She finds it essential for growth of the rat. Its relation to the similar factors postulated by others has been discussed in earlier chapters.

The term "vitamin H" has also been used at times by Paul György [14] to describe one of the fractions concerned in rat dermatitis. A recent German work has included an entire section under the heading of "vitamin H." [15] In this summary Stepp and his co-authors define H as the dialyzable, heat stable substance which is set free from liver proteins by digestions and proteolytic enzymes, according to the observations of György. They assume that this essential is needed by the rat, the chicken and man. There seems little justification for including all these diverse discoveries under this common name. Furthermore, one wonders why the term H should be used to describe the growth factor of Williams, Lewis or Hunt. Apparently the Germans are already marketing some preparations supposed to be concentrates of this factor under the names "Huco Salve" and "Murmil."

MISCELLANEOUS GROWTH FACTORS

The literature dealing with lower forms of life teems with unnamed and ill defined vitamins. There are fewer such factors described for the vertebrates that are popular with orthodox students of nutrition; namely, the rat and the chicken. Since most of these have been considered in other papers in this series, only brief mention of them need be made here.

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[12] McCay, C. M.: The Biochemistry of Fish, Ann. Rev. Biochem. **6**: 445, 1937.

[13] Booher, Lela E.: The Concentration and Properties of Vitamin H, J. Biol. Chem. **119**: 223 (June) 1937.

[14] György, Paul: Stoffwechsel und Immunbiologie der Haut, Handbuch der Kinderheilkunde (Pfaundler-Schlossmann), Berlin **10**: 45-81, 1935.

[15] Stepp, W.; Kühnau, J., and Schroeder, H.: Die Vitamine und ihre klinische Anwendung, Stuttgart, Ferdinand Enke, 1937.

The growth factor for chicks described by Arnold and his associates[16] has proved to be the amino acid arginine, thus accounting for the growth stimulation by such products as peanut meal, liver residue and soy bean meal.

In 1931 Guha[17] found that there were certain factors in milk, grass, spinach and egg yolk that stimulated the growth of rats fed B fractions in the form of vitamin B_1 and autoclaved marmite. To preserve these fractions he found it essential to dry the products at 35 C. in front of a fan. The potency of these materials was lost in both fractions through extraction with ether.

Hogan and his co-workers[18] have shown the need of herbivorous species for some factor that is present in plant juice or alfalfa. During the past two years, evidence has also accumulated in the Animal Nutrition Laboratory at Cornell that lambs require some factor, other than the recognized vitamins, that is present in freshly sprouted oats. Kohler and his co-workers[19] noted the growth stimulating properties of grass juice.

In 1929 Coward and her associates[20] found that some factor was contained in certain preparations of casein and was deficient in others. When a particular sample of casein proved deficient it could be supplemented satisfactorily by milk, lettuce, liver or wheat embryo. These studies were made with rats in the course of other assays.

Liver has provided a fertile source of growth stimulants. It has long been recognized that the essential constituent for brook trout, factor H, is richest in beef liver. Likewise the vitamin requirements of such lower forms as planaria are completely satisfied by liver. The past ten years has indicated that liver has several essential factors, and there is little doubt that many will be discovered as new species are considered by students of nutrition.

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[16] Arnold, Aaron; Kline, O. L.; Elvehjem, C. A., and Hart, E. B.: Further Studies on the Growth Factor Required by Chicks, J. Biol. Chem. **116:** 699 (Dec.) 1936.

[17] Guha, B. C.: Observations on Certain Factors Necessary for the Normal Nutrition of the Rat, Biochem. J. **25:** 960 (No. 3) 1931.

[18] Hogan, A. G., and Johnson, S. R.: Plant Extracts in the Nutrition of Guinea Pigs and Rabbits, Proc. Soc. Exper. Biol. & Med. **35:** 217-221 (Nov.) 1936.

[19] Kohler, G. O.; Elvehjem, C. A., and Hart, E. B.: Growth Stimulating Properties of Grass Juice, Science **83:** 445 (May 8) 1936.

[20] Coward, Katharine H.; Key, Kathleen M., and Morgan, Barbara G. E.: Some Evidence of the Existence of a Further Factor Necessary for the Growth of the Rat, Biochem. J. **23:** 695 (No. 4) 1929.

In 1932 Mapson[21] postulated the existence of a growth factor in liver. It was soluble in 90 per cent alcohol and also in water after the liver was subjected to digestion with papain. This factor was named "physin." Seegers and his co-workers[22] also found that liver contained a growth factor. This tended to be lost on heating. Liver heated at 100 C. for two weeks lost this capacity to stimulate the growth of rats, although the biologic value remained unchanged as far as the protein was concerned.

Elvehjem and his associates[23] have postulated several new factors. Among them is included one for stimulating the growth of rats. They found this in the alcohol-ether precipitate from water extract of hog's liver. They find this is heat labile, so it falls among the thermolabile B factors and needs no further discussion here, other than to note it with the other factors of liver. This factor has recently been christened W by Frost and Elvehjem.[24]

Halliday and Evans[25] attempted to confirm these observations of Elvehjem and his co-workers but came to the conclusion that the precipitate from hog's liver was carrying down vitamin B_6. Therefore they decided that there was inadequate evidence to postulate such a new factor.

The "Y factor" described by Chick and Copping[26] also is found in liver as well as in yeast and its water extracts. This factor is one of the B fractions needed for growth by the rat. Its nature is gradually becoming clear in the course of such thorough studies as those of Edgar and Macrae.[27] These authors find another factor adsorbed on fullers' earth which is neither vitamin B_6 nor riboflavin.

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21. Mapson, L. W.: A New Dietary Principle in Liver, Biochem. J. **26**: 970 (Nov. 4) 1932.

22. Seegers, W. H., and Mattill, H. A.: The Nutritive Value of Beef Heart, Kidney, Round and Liver After Heating and After Alcohol Extraction, J. Nutrition **10**: 271 (Sept.) 1935.

23. Elvehjem, C. A.; Koehn, C. J., Jr., and Oleson, J. J.: An Essential Dietary Factor Found in Yeast and Liver Extract Distinct from Vitamin B_1, B_2, B_4 and Flavin, J. Biol. Chem. **114**: xxxi, 1936; A New Essential Dietary Factor, ibid. **115**: 707 (Oct.) 1936.

24. Frost, D. V., and Elvehjem, C. A.: Further Studies on Factor W, Proc. Soc. Biol. Chem. **31**: 34, 1937.

25. Halliday, Nellie, and Evans, H. M.: On the Claim for a New Essential Dietary Factor in Mammalian Liver, J. Nutrition **14**: 45 (July) 1937.

26. Chick, Harriette, and Copping, Alice M.: The Composite Nature of the Water Soluble Vitamin B_1, Biochem. J. **24**: 1764 (No. 6) 1930.

27. Edgar, Constance E., and Macrae, T. F.: Essential Dietary Factors for the Rat Present in Autoclaved Yeast in Addition to Lactoflavin, Biochem. J. **31**: 886 (June) 1937.

Among the liver extracts must also be included the filtrate factor of Lepkovsky and Jukes,[28] which seems potent in curing dermatitis in chicks, blacktongue in dogs and pellagra in man. This may be identical with the "Y factor," already described, and nicotinic acid, to be discussed later.

In order to rear chickens on synthetic diets, Hogan and his co-workers [29] had to supplement the basal diet with wheat germ oil, acid hydrolyzed yeast, tikitiki and liver extract in addition to carotene and irradiated ergosterol. One can only speculate concerning the undiscovered factors in such an array of supplements.

In 1928 Hunt [30] discovered that there were at least three factors in yeast that were essential for the growth of the rat.

In the course of growth studies with rats, Williams and Lewis [31] also found a factor left in yeast residues after extraction with water and alcohol. This growth factor could not be extracted with any solvent. No later work indicates its relation to the new B fractions.

Vitamin I, or B_7, is the factor postulated by Centanni.[32] He found that an alcoholic extract of rice polishings prevented digestive disturbances in pigeons but did not prevent the symptoms of polyneuritis. Methyl alcohol was the best solvent among the alcohols. Ethyl alcohol was effective at from 95 to 100 per cent but extracted only part of this factor at a 70 per cent concentration.

This I factor recalls the claims of Rosedale [33] made ten years ago. At that time he precipitated the water extract of rice polishings with lead acetate and carried down a factor that permitted evacuation of the bowels and checked the constipation associated with polyneuritis in birds.

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[28] Lepkovsky, Samuel, and Jukes, T. H.: The Effect of Some Reagents on the Filtrate Factor, a Water-Soluble Vitamin Belonging to the Vitamin B Complex and Preventing a Dietary Dermatitis in Chicks, J. Biol. Chem. **114**: 109 (May) 1935. Fouts, P. J.; Lepkovsky, Samuel; Helmer, O. M., and Jukes, T. H.: Successful Treatment of Human Pellagra with the "Filtrate Factor," Proc. Soc. Exper. Biol. & Med. **35**: 345 (Nov.) 1936.

[29] Hogan, A. G.; Boucher, R. V., and Kempster, H. L.: Simplified Rations for the Chick, J. Nutrition **10**: 535 (Nov.) 1935.

[30] Hunt, C. H.: Further Evidence of the Complex Nature of Vitamin B: I. Evidence that a Third Factor Exists, J. Biol. Chem. **79**: 723 (Oct.) 1928.

[31] Williams, G. Z., and Lewis, R. C.: Evidence for the Presence of a Third Factor in the Vitamin B Complex of Yeast, J. Biol. Chem. **89**: 275 (Nov.) 1930.

[32] Centanni, E.: The "Enteral" Vitamin I-B_7 Regulating Digestive Functions of Pigeons, Biochim. e terap. sper. **22**: 137 (April 30) 1935.

[33] Rosedale, J. R.: Preliminary Note on Possible Factor, Biochem. J. **21**: 1266 (No. 6) 1927.

This also recalls the factor of Carter.[34] He found that the heart rate slowed down when pigeons were fed polished rice but that recovery was much slower from feeding B_1 than from such feedstuffs as yeast and wheat. All these factors are difficult to evaluate, since polished rice is such an unbalanced diet in many respects.

Factor J is the term applied by von Euler and Malmberg[35] to a substance found in fruit, especially St. John's berries. This factor seems to cure pneumonia in guinea pigs, while cevitamic acid will not.

VITAMIN K AND OTHER BLOOD CLOTTING FACTORS

Vitamin K is a specific fat soluble substance the absence of which in the diet of chickens causes the blood to become very slow in clotting. Thus far no evidence has indicated its importance for other species, although attempts to study the requirements of other species have been very limited.

The observations that led to this discovery were made by Dam[36] while attempting to determine whether hens could synthesize cholesterol. At this time he was feeding a synthetic diet composed of casein, starch, salt, marmite, filter paper and cod liver oil. In earlier studies, using a similar diet, he had observed subcutaneous and intramuscular hemorrhages as well as erosion of the gizzard. He had also noted that these chickens were anemic and that the number of erythrocytes was only about two million instead of three or more. In 1930 Horvath[37] also observed that the coagulation of the blood of chickens was accelerated by diets of sprouted soy beans.

The same hemorrhagic condition described by Dam was reported by McFarlane and his associates[38] in 1931 when they were attempting to establish the need of chickens for fat soluble vitamins. These workers were employing diets of about 70 per cent rice and 15 per cent marmite supplemented with various protein sources such as fish meal and casein. They suffered heavy losses from hemorrhage in chickens fed on a diet containing ether extracted fish meal but no losses if the fish meal was not extracted. Further, they found that blood from chickens fed this extracted fish meal could set in the laboratory over night without clotting.

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[34] Carter, C. W.: Heart Block in Pigeons, Curative Factor, Biochem. J. **24:** 1811 (No. 6) 1930.

29: 5890.

[36] Dam, Henrik: Cholesterol Synthesis in the Animal Body, Biochem. Ztschr. **220:** 158, 1930; Cholesterol Metabolism in Chickens and Chicken Eggs, ibid. **215:** 475, 1929.

[37] Horvath, A. A.: Changes in Hens' Blood Produced by a Diet of Sprouted Soy Beans, Am. J. Physiol. **94:** 65-68 (July) 1930.

[38] McFarlane, W. D.; Graham, W. R., and Richardson, Frederick: The Fat Soluble Vitamin Requirements of the Chick: I. The Vitamin A and Vitamin D Content of Fish Meal and Meat Meal, Biochem. J. **25:** 358 (No. 1) 1931.

In 1934 Dam and Schønheyder[39] found that this disease, which resembled scurvy in some respects, developed if chicks were fed cereals but that otherwise it developed even if the diet contained 15 per cent yeast. Neither lemon juice nor cevitamic acid alleviated the symptoms.

In the course of further studies it was found that the fats of hog's liver and hemp seed were rich in the factor while the fats from rye, sunflower, corn and rice had no effect. Cod liver oil was also without effect.

Dam and his co-workers[40] later found that the normal amount of prothrombin was lacking in chickens fed a diet deficient in vitamin K. The condition could be developed in about three weeks on a diet of casein 10, ether extracted pig's liver 10, dried yeast 15, sucrose 62.3, salt mixture 2.7 and cod liver oil 4. Three days after vitamin K is fed to deficient animals the blood becomes normal.

Rats and guinea pigs were fed a diet similar to that used for the chickens but no symptoms developed. A man suffering from hemophilia also yielded no response to the feeding of concentrates of the vitamin.

The factor was concentrated by Almquist[41] in 1936 so that 2 mg. formed a sufficient supplement for 1 Kg. of feed. This concentrate was prepared from a hexane extract of alfalfa. By distillation this material was concentrated still further. Under very low pressures the potent material could be distilled at from 120 to 140 C. This vitamin is unstable to alcoholic alkali and is unsaturated as well as colorless. It has no sulfur or phosphorus but a small amount of nitrogen. Color tests indicate an indole nucleus.

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[39] Dam, Henrik, and Schønheyder, Fritz: A Deficiency Disease in Chicks Resembling Scurvy, Biochem. J. **28**: 1355 (No. 4) 1934.

[40] Dam, Henrik: The Antihemorrhagic Vitamin of the Chick, Nature **135**: 652, 1935; Biochem. J. **29**: 1273 (June) 1935. Schønheyder, Fritz: Measurement and Biological Action, Nature **135**: 652, 1935. Dam, Henrik; Schønheyder, Fritz, and Tage-Hansen, Erik: Studies of the Mode of Action of Vitamin K, Biochem. J. **30**: 1075 (June) 1936. Schønheyder, Fritz: The Quantitative Determination of Vitamin K, ibid. **30**: 890 (May) 1936. Dam, Henrik, and Schønheyder, Fritz: Vitamin K, Nord. med. tidskr. **12**: 1097 (July 4) 1936.

[41] Almquist, H. J.: Purification of the Antihemorrhagic Vitamin, J. Biol. Chem. **114**: 241 (May) 1936; Purification of the Antihemorrhagic Vitamin by Distillation, ibid. **115**: 589 (Sept.) 1936; Chemical and Physical Studies on the Antihemorrhagic Vitamin, ibid. **117**: 517 (Feb.) 1937. Almquist, H. J., and Stokstad, E. L. R.: Factors Influencing the Incidence of Dietary Hemorrhagic Disease in Chicks, J. Nutrition **12**: 329 (Oct.) 1936.

This vitamin seems to be synthesized by bacterial action, since its amount tends to increase in feedstuffs subjected to such action. The amount also increases in the feces of chicks on the deficient diet if these stand for a day. The diet of the hen may also influence the development of the disease in chicks.

Vitamin K seems to be essential for the synthesis of prothrombin in the case of man, dog and rat in addition to the chicken. In bile fistula rats Greaves and Schmidt [41a] made the important observation that the clotting time was increased very much when the prothrombin level of the blood dropped to a level of 20-30 per cent of the normal. This level of prothrombin was raised by feeding 2-3 cc. of beef bile daily or massive doses of vitamin K. The best results were obtained by combining bile and vitamin K. They concluded that bile acted as the carrying agent for the passage of vitamin K across the intestinal wall. Their data indicate that this is an essential for rats.

In 1935 Hawkins and Whipple [41b] observed the bleeding tendency in dogs after long standing biliary fistulas. They noted that this was partly corrected by feeding bile. In a series of reports Smith, Warner, Brinkhous and Seegers [41c] have shown that this bleeding in dogs takes place after the prothrombin reaches a level of about ten per cent. Using a diet that was relatively low in vitamin K, they were able to increase the prothrombin slowly by feeding bile. The increase became very rapid, however, when they fed a concentrate of vitamin K prepared from alfalfa as well as the bile. Thus the dog seems to need vitamin K. In this species as well as in the rat bile seems to function in the passage from the food into the circulation.

41a. Greaves, J. D., and Schmidt, C. L. A.: Nature of the Factor Concerned in Loss of Blood Coagulability of Bile Fistula Rats, Proc. Soc. Exper. Biol. & Med. **37**: 43 (Oct.) 1937.

41b. Hawkins, W. B., and Whipple, G. H.: Bile Fistulas and Related Abnormalities, J. Exper. Med. **62**: 599 (Oct. 1) 1935; Prothrombin Deficiency the Cause of Bleeding in Bile Fistula Dogs, **63**: 795 (June 1) 1936.

41c. Smith, H. P.; Warner, E. D.; Brinkhous, K. M., and Seegers, W. H.: Bleeding Tendency and Prothrombin Deficiency in Biliary Fistula Dogs. Effect of Feeding Bile and Vitamin K, J. Exper. Med. **67**: 911 (June) 1938.

Brinkhous and co-workers [41d] have also fed vitamin K from alfalfa mixed with sodium taurocholate to cases of obstructive jaundice. The same results were obtained as in the experiments with dogs except in cases with extensive liver damage. These failed to respond. For their vitamin K concentrate they extracted alfalfa leaf meal with petroleum ether (Skellysolve A) and then drove off the solvent. They fed 200-400 mg. of this residue with two per cent of sodium taurocholate daily. Their findings were confirmed independently by Butt, Snell and Osterberg.[41e]

Vitamin K seems to be essential for a number of species including man. Whether it functions in furnishing an essential fragment for the building of prothrombin or whether it acts indirectly upon the liver cells is unknown.

About 1931 claims were made that vitamin A was concerned with the clotting of blood. As purer preparations became available, however, this was disproved. Recently, however, Schiff and Hirschberger [42] have found that there is some factor, which they term T, in egg yolk and sesame oil. This seems to increase the platelet count in both human and rat blood. They found that from five to ten drops of sesame oil per day or five egg yolks affords ample of this essential for treating children. It has also been claimed that semifasting dogs develop a severe thrombopenia and need this essential. Evidence is not clear that factor T and vitamin K are related.

The failure of blood to coagulate in "sweet clover disease" has long been recognized, especially in young cattle that have consumed partly spoiled clover.[43] Quick [44] finds that this disease can be reproduced in rabbits. It is cured by feeding alfalfa. However, this disease is not related to the vitamin other than that alfalfa probably acts in furnishing materials for the synthesis of prothrombin. Hogan[18] also observed that the ether extract of alfalfa prevented the hemorrhages of the nursing young in the case of rabbits.

[41d] Brinkhous, K. M.; Smith, H. P., and Warner, E. D.: Prothrombin Deficiency and the Bleeding Tendency in Obstructive Jaundice and in Biliary Fistula. Effect of Feeding Bile and Alfalfa (Vitamin K), Am. J. Med. Sc. **196:** 50 (July) 1938.

[41e] Butt, H. R.; Snell, A. M., and Osterberg, A. E.: Proc. Staff Meet., Mayo Clin. **13:** 74 (Feb. 7) 1938.

[42] Schiff, E., and Hirschberger, C.: Weitere untersuchungen zur Frage des Morbus maculosus Werlhofi: V. A-Vitamin and Thrombozytose, Jahrb. f. Kinderh. **147:** 81 (July) 1936.

[43] Morrison, F. B.: Feeds and Feeding, ed. 20, Ithaca, N. Y., Morrison Publishing Company, 1936.

[44] Quick, A. J.: The Coagulation Defect in Sweet Clover Disease and in the Hemorrhagic Disease of Dietary Origin, Am. J. Physiol. **118:** 260 (Feb.) 1937.

It is well known that some fish meals are injurious to animals. Scott and Cook[45] find that this injury is due both to the deficiency of vitamin K and to toxic factors in the fish meal. The toxic factor is chiefly reflected in the distorted blood picture with marked increase in lymphocytes and leukocytes.

GIZZARD EROSION FACTOR

The erosion of the gizzard was observed early in the studies of Dam. The question of whether or not this is due to a specific deficiency is still unsettled. Almquist and Stokstad[46] found that it was cured by a saponifiable fraction of the hexane extract of alfalfa or dried kale. Cod liver oil, wheat germ oil, orange oil, egg yolk and alfalfa ash failed.

Bird and his associates[47] seemed to feel that the factor was specific but its properties, as far as solvents are concerned, conflicted with those found by Almquist. Thus the Wisconsin workers found the factor in such materials as pork lungs, liver and kidneys but they were not able to extract it with water, alcohol or ether. They found it to be labile to both dry heat and autoclaving at 120 C. Among the grains they found oats the best. In their hands alfalfa was not potent. Further studies will undoubtedly be needed to determine whether this is a specific factor.

VITAMIN P

Vitamin P is the term applied by Rusznyák and Szent-Györgyi[48] to a substance that occurs in lemon juice and red peppers. In certain pathologic conditions characterized by increased permeability of the capillary wall, they found injection of concentrates to be effective when cevitamic acid failed. The early work indicated that the effective agent belonged to the vegetable dyes of the flavine group.

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[45] Scott, K. G., and Cook, S. F.: The Syndrome Induced in Poultry by an Intoxication Factor and Its Relation to the Antihemorrhagic Factor, Univ. Calif. Pub. Physiol. **8**: 135, 1936.

[46] Almquist, H. J., and Stokstad, E. L. R.: A Nutritional Deficiency Causing Gizzard Erosions in Chicks, Nature **137**: 581 (April 4) 1936.

[47] Bird, H. R.; Kline, O. L.; Elvehjem, C. A.; Hart, E. B., and Halpin, J. G.: The Distribution and Properties of the Antigizzard Erosion Factor Required by Chicks, J. Nutrition **12**: 571 (Dec.) 1936.

[48] Rusznyák, S., and Szent-Györgyi, Albert: Vitamin-P Flavonols as Vitamins, Nature **138**: 27 (July 4) 1936. Bentsáth, A.; Rusznyák, S., and Szent-Györgyi, Albert: Vitamin Nature of Flavones, ibid. **138**: 798 (Nov. 7) 1936.

Workers from the same laboratory used guinea pigs that were fed a diet to produce scurvy. They found that these animals finally died of scurvy if fed the concentrate of vitamin P termed "citrin." However, these animals lived for about forty-four days, while the others lived only twenty-eight days.

In a later study Bruckner and Szent-Györgyi[49] found citrin to be a mixture of hesperidine and eriodictyol glucoside and ascribed the activity and color to the latter. In unripe oranges they found much hesperidine but not much of the other compound.

The existence of this "vitamin P" has been questioned by Zilza.[50] He presents evidence which, as far as the experiments on guinea pigs are concerned, indicates that there is no reason for postulating a new factor. His work seems to show that the animals were suffering from vitamin C deficiency, which was relieved to a slight extent by contamination of the "citrin."

NICOTINIC ACID

From time to time nicotinic acid returns among the growth stimulants that resemble vitamins. Thus the Funks[51] have presented evidence recently that the growth of rats and pigeons is stimulated by such derivatives. This work is interesting in the light of the need of staphylococcus for the same essential as a supplement to B_1 as reviewed by Kögl.[7] Furthermore Elvehjem and his co-workers[52] have found that nicotinic acid will cure blacktongue in dogs. These workers have isolated considerable amounts of crystalline nicotine amide from active liver fractions. The effectiveness of this compound in the treatment of human pellagra has now been established and is discussed elsewhere.

TOXIC SUBSTANCES

The specific toxicities of various foodstuffs and the counter action of other vitamin-like components of the diet cannot be neglected in a discussion of vitamins, although the subject material is too extensive for since hydrogenation of cod liver oil causes it to lose this toxic action. No explanation has been offered concerning the method by which such substances as alfalfa and yeast can react with an unsaturated fatty acid to remove its toxic action.

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[49] Bruckner, V., and Szent-Gyorgyi, Albert: Chemical Nature of Citrin, Nature **138**: 1057 (Dec. 19) 1936.

[50] Zilva, S. S.: Vitamin P, Biochem. J. **31**: 915 (June) 1936.

[51] Funk, Casimir, and Funk, I. C.: The Value of Pyridine Derivaives in Nutrition, Proc. Soc. Biol. Chemists **31**: 35, 1937.

[52] Elvehjem, C. A.; Madden, R. J.; Strong, F. M., and Woolley, D. W.: Relation of Nicotinic Acid and Nicotinic Acid Amide to Canine Blacktongue, J. Am. Chem. Soc. **59**: 1767, 1937.

Hilditch and Thompson[62] have produced evidence indicating that the unsaturated fatty acids of the C_{20}-C_{22} series may account for part of the toxic action of cod liver oil. This is probably only part of the explanation, however, because salmon oil, which is rich in these same compounds, fails to produce the consistent effect of cod liver oil in lowering the secretion of milk fat. No mechanism has been devised to explain the interplay between dietary constituents in the case either of poisoning from egg white or of that from cod liver oil.

Finally a third field that has been given considerable attention in recent years is that concerned with the cerebellar disorder in chicks first discovered by Pappenheimer and Goettsch.[63] They found this to be produced in chicks fed a diet of milk powder, casein, starch, yeast, cod liver oil, salts and filter paper. In ducks fed a similar diet, injury to the muscles developed with no effect on the brain,[64] an observation of special interest because it shows that a science of nutrition cannot be grounded on one or two species such as the rat and the chicken. In later studies, vegetable oils, incorporated in the diet at 20 per cent levels, were found to afford complete protection in the case of chicks.[65]

Jungherr and Pappenheimer have found more recently that turkeys respond to this diet in a still different manner. This species develops selective necrosis of the smooth muscle of the gizzard wall.[66]

CONCLUSIONS

In such a review as this, which by its nature covers much of the vitamin literature that has not been amply confirmed, one must recognize that it will become obsolete in part, at least, by the time it is printed. However, certain trends are clear. The science of nutrition is slowly extending to include more animal species. Each species that is included leads to the discovery of new vitamin requirements and specific reactions to mixtures of feedstuff. However, this broadening of the base must lead to a more complete science capable of providing generalizations with more certainty. Finally the need for better systems of naming new vitamins is obvious. The biologist who feeds animals must inevitably precede the chemist in the discovery of new essentials. The isolation of new factors is slow because these factors depend on biologic testing as well as involved technics. For this reason it is likely that there will be an increasing accumulation of vitamins awaiting the organic chemist. A better system of numbering them must be devised to prevent confusion in the period between their discovery and their isolation. Possibly some numbering system issued from a central office such as that of the League of Nations must be resorted to in the near future.[67]

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[62] Hilditch, T. P., and Thompson, H. M.: The Effect of Certain Ingested Fatty Oils upon the Composition of Milk Fat, Biochem. J. **30:** 677 (No. 4) 1936.

[63] Pappenheimer, A. M., and Goettsch, Marianne: A Cerebellar Disorder in Chicks, Apparently of Nutritional Origin, J. Exper. Med. **53:** 11 (Jan.) 1931.

[64] Pappenheimer, A. M., and Goettsch, Marianne: Nutritional Myopathy in Ducklings, J. Exper. Med. **59:** 35 (Jan.) 1934.

[65] Pappenheimer, A. M., and Goettsch, Marianne: Protection Afforded by Certain Vegetable Oils Against Nutritional Encephalomalacia of Chicks, Proc. Soc. Exper. Biol. & Med. **31:** 777 (April) 1934.

[66] Jungherr, E., and Pappenheimer, A. M.: Proc. Soc. Exper. Biol. & Med. **37:** 520-526, 1937.

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[67] McCay, C. M.: Can We Abandon the Vitamin Alphabet? Science **86:** 347 (Oct. 15) 1937.

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